

SUBSTITUTED HETEROCYCLIC COMPOUNDS AND METHODS OF USEBACKGROUND OF THE INVENTION

The present invention comprises a new class of compounds useful in treating
5 diseases, such as TNF- α , IL-1 β , IL-6 and/or IL-8 mediated diseases and other
maladies, such as pain and diabetes. In particular, the compounds of the invention
are useful for the prophylaxis and treatment of diseases or conditions involving
inflammation. This invention also relates to intermediates and processes useful in
the preparation of such compounds.

10 Interleukin-1 (IL-1) and Tumor Necrosis Factor α (TNF- α) are pro-
inflammatory cytokines secreted by a variety of cells, including monocytes and
macrophages, in response to many inflammatory stimuli (*e.g.*, lipopolysaccharide -
LPS) or external cellular stress (*e.g.*, osmotic shock and peroxide).

Elevated levels of TNF- α and/or IL-1 over basal levels have been implicated
15 in mediating or exacerbating a number of disease states including rheumatoid
arthritis; Pagets disease; osteoporosis; multiple myeloma; uveitis; acute and
chronic myelogenous leukemia; pancreatic β cell destruction; osteoarthritis;
rheumatoid spondylitis; gouty arthritis; inflammatory bowel disease; adult
respiratory distress syndrome (ARDS); psoriasis; Crohn's disease; allergic rhinitis;
20 ulcerative colitis; anaphylaxis; contact dermatitis; asthma; muscle degeneration;
cachexia; Reiter's syndrome; type I and type II diabetes; bone resorption diseases;
graft vs. host reaction; ischemia reperfusion injury; atherosclerosis; brain trauma;
multiple sclerosis; cerebral malaria; sepsis; septic shock; toxic shock syndrome;
fever, and myalgias due to infection. HIV-1, HIV-2, HIV-3, cytomegalovirus
25 (CMV), influenza, adenovirus, the herpes viruses (including HSV-1, HSV-2), and
herpes zoster are also exacerbated by TNF- α .

It has been reported that TNF- α plays a role in head trauma, stroke, and
ischemia. For instance, in animal models of head trauma (rat), TNF- α levels
increased in the contused hemisphere (Shohami et al., *J. Cereb. Blood Flow Metab.*
30 14, 615 (1994)). In a rat model of ischemia wherein the middle cerebral artery was
occluded, the levels of TNF- α mRNA of TNF- α increased (Feurstein et al.,
Neurosci. Lett. 164, 125 (1993)). Administration of TNF- α into the rat cortex has

been reported to result in significant neutrophil accumulation in capillaries and adherence in small blood vessels. TNF- α promotes the infiltration of other cytokines (IL-1 β , IL-6) and also chemokines, which promote neutrophil infiltration into the infarct area (Feurstein, *Stroke* 25, 1481 (1994)). TNF- α has also been
5 implicated to play a role in type II diabetes (Endocrinol. 130, 43-52, 1994; and Endocrinol. 136, 1474-1481, 1995).

TNF- α appears to play a role in promoting certain viral life cycles and disease states associated with them. For instance, TNF- α secreted by monocytes induced elevated levels of HIV expression in a chronically infected T cell clone
10 (Clouse et al., *J. Immunol.* 142, 431 (1989)). Lahdevirta et al., (*Am. J. Med.* 85, 289 (1988)) discussed the role of TNF- α in the HIV associated states of cachexia and muscle degradation.

TNF- α is upstream in the cytokine cascade of inflammation. As a result, elevated levels of TNF- α may lead to elevated levels of other inflammatory and
15 proinflammatory cytokines, such as IL-1, IL-6, and IL-8.

Elevated levels of IL-1 over basal levels have been implicated in mediating or exacerbating a number of disease states including rheumatoid arthritis; osteoarthritis; rheumatoid spondylitis; gouty arthritis; inflammatory bowel disease; adult respiratory distress syndrome (ARDS); psoriasis; Crohn's disease; ulcerative
20 colitis; anaphylaxis; muscle degeneration; cachexia; Reiter's syndrome; type I and type II diabetes; bone resorption diseases; ischemia reperfusion injury; atherosclerosis; brain trauma; multiple sclerosis; sepsis; septic shock; and toxic shock syndrome. Viruses sensitive to TNF- α inhibition, e.g., HIV-1, HIV-2, HIV-3, are also affected by IL-1.

25 TNF- α and IL-1 appear to play a role in pancreatic β cell destruction and diabetes. Pancreatic β cells produce insulin which helps mediate blood glucose homeostasis. Deterioration of pancreatic β cells often accompanies type I diabetes. Pancreatic β cell functional abnormalities may occur in patients with type II diabetes. Type II diabetes is characterized by a functional resistance to insulin.
30 Further, type II diabetes is also often accompanied by elevated levels of plasma glucagon and increased rates of hepatic glucose production. Glucagon is a

regulatory hormone that attenuates liver gluconeogenesis inhibition by insulin. Glucagon receptors have been found in the liver, kidney and adipose tissue. Thus glucagon antagonists are useful for attenuating plasma glucose levels (WO 97/16442, incorporated herein by reference in its entirety). By antagonizing the glucagon receptors, it is thought that insulin responsiveness in the liver will improve, thereby decreasing gluconeogenesis and lowering the rate of hepatic glucose production.

In rheumatoid arthritis models in animals, multiple intra-articular injections of IL-1 have led to an acute and destructive form of arthritis (Chandrasekhar et al., *Clinical Immunol Immunopathol.* 55, 382 (1990)). In studies using cultured rheumatoid synovial cells, IL-1 is a more potent inducer of stromelysin than is TNF- α (Firestein, *Am. J. Pathol.* 140, 1309 (1992)). At sites of local injection, neutrophil, lymphocyte, and monocyte emigration has been observed. The emigration is attributed to the induction of chemokines (e.g., IL-8), and the up-regulation of adhesion molecules (Dinarello, *Eur. Cytokine Netw.* 5, 517-531 (1994)).

IL-1 also appears to play a role in promoting certain viral life cycles. For example, cytokine-induced increase of HIV expression in a chronically infected macrophage line has been associated with a concomitant and selective increase in IL-1 production (Folks et al., *J. Immunol.* 136, 40 (1986)). Beutler et al. (*J. Immunol.* 135, 3969 (1985)) discussed the role of IL-1 in cachexia. Baracos et al. (*New Eng. J. Med.* 308, 553 (1983)) discussed the role of IL-1 in muscle degeneration.

In rheumatoid arthritis, both IL-1 and TNF- α induce synoviocytes and chondrocytes to produce collagenase and neutral proteases, which leads to tissue destruction within the arthritic joints. In a model of arthritis (collagen-induced arthritis (CIA) in rats and mice), intra-articular administration of TNF- α either prior to or after the induction of CIA led to an accelerated onset of arthritis and a more severe course of the disease (Brahm et al., *Lymphokine Cytokine Res.* 11, 253 (1992); and Cooper, *Clin. Exp. Immunol.* 898, 244 (1992)).

IL-8 has been implicated in exacerbating and/or causing many disease states in which massive neutrophil infiltration into sites of inflammation or injury (e.g.,

ischemia) is mediated by the chemotactic nature of IL-8, including, but not limited to, the following: asthma, inflammatory bowel disease, psoriasis, adult respiratory distress syndrome, cardiac and renal reperfusion injury, thrombosis and glomerulonephritis. In addition to the chemotaxis effect on neutrophils, IL-8 also
5 has the ability to activate neutrophils. Thus, reduction in IL-8 levels may lead to diminished neutrophil infiltration.

Several approaches have been taken to block the effect of TNF- α . One approach involves using soluble receptors for TNF- α (e.g., TNFR-55 or TNFR-75), which have demonstrated efficacy in animal models of TNF- α -mediated disease
10 states. A second approach to neutralizing TNF- α using a monoclonal antibody specific to TNF- α , cA2, has demonstrated improvement in swollen joint count in a Phase II human trial of rheumatoid arthritis (Feldmann et al., *Immunological Reviews*, pp. 195-223 (1995)). These approaches block the effects of TNF- α and IL-1 by either protein sequestration or receptor antagonism.

15 US 5,100,897, incorporated herein by reference in its entirety, describes pyrimidinone compounds useful as angiotensin II antagonists wherein one of the pyrimidinone ring nitrogen atoms is substituted with a substituted phenylmethyl or phenethyl radical.

US 5,162,325, incorporated herein by reference in its entirety, describes
20 pyrimidinone compounds useful as angiotensin II antagonists wherein one of the pyrimidinone ring nitrogen atoms is substituted with a substituted phenylmethyl radical.

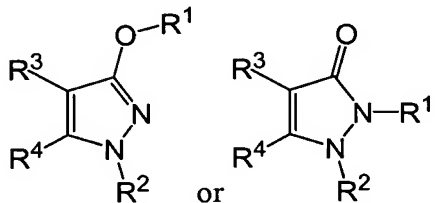
EP 481448, incorporated herein by reference in its entirety, describes
25 pyrimidinone compounds useful as angiotensin II antagonists wherein one of the pyrimidinone ring nitrogen atoms is substituted with a substituted phenyl, phenylmethyl or phenethyl radical.

CA 2,020,370, incorporated herein by reference in its entirety, describes
30 pyrimidinone compounds useful as angiotensin II antagonists wherein one of the pyrimidinone ring nitrogen atoms is substituted with a substituted biphenylaliphatic hydrocarbon radical.

BRIEF DESCRIPTION OF THE INVENTION

The present invention comprises a new class of compounds useful in the prophylaxis and treatment of diseases, such as TNF- α , IL-1 β , IL-6 and/or IL-8 mediated diseases and other maladies, such as pain and diabetes. In particular, the compounds of the invention are useful for the prophylaxis and treatment of diseases or conditions involving inflammation. Accordingly, the invention also comprises pharmaceutical compositions comprising the compounds, methods for the prophylaxis and treatment of TNF- α , IL-1 β , IL-6 and/or IL-8 mediated diseases, such as inflammatory, pain and diabetes diseases, using the compounds and compositions of the invention, and intermediates and processes useful for the preparation of the compounds of the invention.

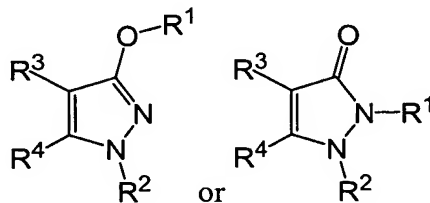
The compounds of the invention are represented by the following general structure:



The foregoing merely summarizes certain aspects of the invention and is not intended, nor should it be construed, as limiting the invention in any way. All patents and other publications recited herein are hereby incorporated by reference in their entirety.

DETAILED DESCRIPTION OF THE INVENTION

In accordance with the present invention, there is provided compounds of the formula:



or a pharmaceutically acceptable salt thereof, wherein

R¹ is H or C₁₋₈alkyl;

R² is C₁₋₈alkyl, phenyl, benzyl, R^c, R^f, C₁₋₄alkylR^c, C₁₋₄alkylR^f or R^g;

R^3 is phenyl, naphthyl, or a saturated or unsaturated 5- or 6-membered ring heterocycle containing 1-4 heteroatoms selected from N, O and S, wherein no more than 2 of the heteroatoms are O or S, and the heterocycle is substituted by 0, 1 or 2 oxo groups and is optionally fused with a benzo group, any of which are substituted

5 by 0, 1, 2 or 3 substituents selected from C_{1-8} alkyl, C_{1-4} haloalkyl, halo, cyano, nitro, $-C(=O)R^b$, $-C(=O)OR^b$, $-C(=O)NR^aR^a$, $-C(=NR^a)NR^aR^a$, $-OR^a$, $-OC(=O)R^b$, $-OC(=O)NR^aR^a$, $-OC(=O)N(R^a)S(=O)_2R^b$, $-OC_{2-6}alkylNR^aR^a$, $-OC_{2-6}alkylOR^a$, $-SR^a$, $-S(=O)R^b$, $-S(=O)_2R^b$, $-S(=O)_2NR^aR^a$, $-S(=O)_2N(R^a)C(=O)R^b$, $-S(=O)_2N(R^a)C(=O)OR^b$, $-S(=O)_2N(R^a)C(=O)NR^aR^a$, $-NR^aR^a$, $-N(R^a)C(=O)R^b$, $-N(R^a)C(=O)OR^b$, $-N(R^a)C(=O)NR^aR^a$, $-N(R^a)C(=NR^a)NR^aR^a$, $-N(R^a)S(=O)_2R^b$, $-N(R^a)S(=O)_2NR^aR^a$, $-NR^aC_{2-6}alkylNR^aR^a$ and $-NR^aC_{2-6}alkylOR^a$;

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R^4 is phenyl, naphthyl, or a saturated or unsaturated 5- or 6-membered ring heterocycle containing 1-4 heteroatoms selected from N, O and S, wherein no more than 2 of the heteroatoms are O or S, and the heterocycle is substituted by 0, 1 or 2

15 oxo groups and is optionally fused with a benzo group, any of which are substituted by 0, 1, 2 or 3 substituents selected from C_{1-8} alkyl, C_{1-4} haloalkyl, halo, cyano, nitro, $-C(=O)R^b$, $-C(=O)OR^b$, $-C(=O)NR^aR^a$, $-C(=NR^a)NR^aR^a$, $-OR^a$, $-OC(=O)R^b$, $-OC(=O)NR^aR^a$, $-OC(=O)N(R^a)S(=O)_2R^b$, $-OC_{2-6}alkylNR^aR^a$, $-OC_{2-6}alkylOR^a$, $-SR^a$, $-S(=O)R^b$, $-S(=O)_2R^b$, $-S(=O)_2NR^aR^a$, $-S(=O)_2N(R^a)C(=O)R^b$, $-S(=O)_2N(R^a)C(=O)OR^b$, $-S(=O)_2N(R^a)C(=O)NR^aR^a$, $-NR^aR^a$, $-N(R^a)C(=O)R^b$, $-N(R^a)C(=O)OR^b$, $-N(R^a)C(=O)NR^aR^a$, $-N(R^a)C(=NR^a)NR^aR^a$, $-N(R^a)S(=O)_2R^b$, $-N(R^a)S(=O)_2NR^aR^a$, $-NR^aC_{2-6}alkylNR^aR^a$ and $-NR^aC_{2-6}alkylOR^a$;

20

R^a is independently at each instance H or R^b ;

R^b is independently at each instance C_{1-8} alkyl, phenyl or benzyl;

25 R^c is independently at each instance a saturated or unsaturated 5-, 6- or 7-membered monocyclic or 6-, 7-, 8-, 9-, 10- or 11-membered bicyclic ring containing 1, 2 or 3 atoms selected from N, O and S, wherein the ring is fused with 0 or 1 benzo groups and 0 or 1 saturated or unsaturated 5-, 6- or 7-membered heterocyclic ring containing 1, 2 or 3 atoms selected from N, O and S; wherein the

30 carbon atoms of the ring are substituted by 0, 1 or 2 oxo groups;

R^d is independently at each instance C_{1-8} alkyl, C_{1-4} haloalkyl, halo, cyano, nitro, $-C(=O)R^b$, $-C(=O)OR^b$, $-C(=O)NR^aR^a$, $-C(=NR^a)NR^aR^a$, $-OR^a$, $-OC(=O)R^b$,

- OC(=O)NR^aR^a, -OC(=O)N(R^a)S(=O)₂R^b, -OC₂₋₆alkylNR^aR^a, -OC₂₋₆alkylOR^a, -SR^a,
 -S(=O)R^b, -S(=O)₂R^b, -S(=O)₂NR^aR^a, -S(=O)₂N(R^a)C(=O)R^b,
 -S(=O)₂N(R^a)C(=O)OR^b, -S(=O)₂N(R^a)C(=O)NR^aR^a, -NR^aR^a, -N(R^a)C(=O)R^b,
 -N(R^a)C(=O)OR^b, -N(R^a)C(=O)NR^aR^a, -N(R^a)C(=NR^a)NR^aR^a, -N(R^a)S(=O)₂R^b,
 5 -N(R^a)S(=O)₂NR^aR^a, -NR^aC₂₋₆alkylNR^aR^a or -NR^aC₂₋₆alkylOR^a;

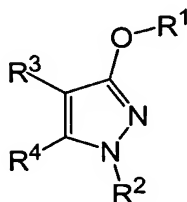
R^c is independently at each instance C₁₋₆alkyl substituted by 1, 2 or 3 substituents independently selected from R^d;

R^f is independently at each instance R^c substituted by 1, 2 or 3 substituents independently selected from R^d; and

- 10 R^g is independently at each instance R^b substituted by 1, 2 or 3 substituents independently selected from R^c, R^f and R^d.

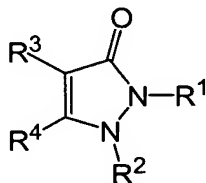
In another embodiment, in conjunction with any of the above or below embodiments, R¹ is H.

- 15 In another embodiment, in conjunction with any of the above or below embodiments, the compound has the structure



wherein R¹ is C₁₋₈alkyl.

In another embodiment, in conjunction with any of the above or below embodiments, the compound has the structure



20

wherein R¹ is C₁₋₈alkyl.

In another embodiment, in conjunction with any of the above or below embodiments, R² is R^c, R^f, C₁₋₄alkylR^c, C₁₋₄alkylR^f or R^g.

- 25 In another embodiment, in conjunction with any of the above or below embodiments, R² is R^c.

In another embodiment, in conjunction with any of the above or below embodiments, R^2 is $C_{1-4}alkylR^c$.

In another embodiment, in conjunction with any of the above or below embodiments, R^2 is $C_{1-4}alkylR^f$.

5 In another embodiment, in conjunction with any of the above or below embodiments, R^2 is R^g .

In another embodiment, in conjunction with any of the above or below embodiments, R^2 is $C_{1-8}alkyl$, phenyl or benzyl.

10 In another embodiment, in conjunction with any of the above or below embodiments, R^2 is $C_{1-8}alkyl$.

In another embodiment, in conjunction with any of the above or below embodiments, R^2 is phenyl or benzyl.

In another embodiment, in conjunction with any of the above or below embodiments, R^3 is phenyl or naphthyl both of which are substituted by 0, 1, 2 or 3
 15 substituents selected from $C_{1-8}alkyl$, $C_{1-4}haloalkyl$, halo, cyano, nitro, $-C(=O)R^b$,
 $-C(=O)OR^b$, $-C(=O)NR^aR^a$, $-C(=NR^a)NR^aR^a$, $-OR^a$, $-OC(=O)R^b$, $-OC(=O)NR^aR^a$,
 $-OC(=O)N(R^a)S(=O)_2R^b$, $-OC_{2-6}alkylNR^aR^a$, $-OC_{2-6}alkylOR^a$, $-SR^a$, $-S(=O)R^b$,
 $-S(=O)_2R^b$, $-S(=O)_2NR^aR^a$, $-S(=O)_2N(R^a)C(=O)R^b$, $-S(=O)_2N(R^a)C(=O)OR^b$,
 $-S(=O)_2N(R^a)C(=O)NR^aR^a$, $-NR^aR^a$, $-N(R^a)C(=O)R^b$, $-N(R^a)C(=O)OR^b$,
 20 $-N(R^a)C(=O)NR^aR^a$, $-N(R^a)C(=NR^a)NR^aR^a$, $-N(R^a)S(=O)_2R^b$, $-N(R^a)S(=O)_2NR^aR^a$,
 $-NR^aC_{2-6}alkylNR^aR^a$ and $-NR^aC_{2-6}alkylOR^a$.

In another embodiment, in conjunction with any of the above or below embodiments, R^3 is unsubstituted naphthyl or phenyl substituted by 1, 2 or 3
 25 substituents selected from $C_{1-8}alkyl$, $C_{1-4}haloalkyl$, halo, cyano, nitro, $-C(=O)R^b$,
 $-C(=O)OR^b$, $-C(=O)NR^aR^a$, $-C(=NR^a)NR^aR^a$, $-OR^a$, $-OC(=O)R^b$, $-OC(=O)NR^aR^a$,
 $-OC(=O)N(R^a)S(=O)_2R^b$, $-OC_{2-6}alkylNR^aR^a$, $-OC_{2-6}alkylOR^a$, $-SR^a$, $-S(=O)R^b$,
 $-S(=O)_2R^b$, $-S(=O)_2NR^aR^a$, $-S(=O)_2N(R^a)C(=O)R^b$, $-S(=O)_2N(R^a)C(=O)OR^b$,
 $-S(=O)_2N(R^a)C(=O)NR^aR^a$, $-NR^aR^a$, $-N(R^a)C(=O)R^b$, $-N(R^a)C(=O)OR^b$,
 $-N(R^a)C(=O)NR^aR^a$, $-N(R^a)C(=NR^a)NR^aR^a$, $-N(R^a)S(=O)_2R^b$, $-N(R^a)S(=O)_2NR^aR^a$,
 30 $-NR^aC_{2-6}alkylNR^aR^a$ and $-NR^aC_{2-6}alkylOR^a$.

In another embodiment, in conjunction with any of the above or below embodiments, R^3 is unsubstituted naphthyl.

In another embodiment, in conjunction with any of the above or below embodiments, R^3 is phenyl substituted by 1, 2 or 3 substituents selected from C_{1-8} alkyl, C_{1-4} haloalkyl, halo, cyano, nitro, $-C(=O)R^b$, $-C(=O)OR^b$, $-C(=O)NR^aR^a$, $-C(=NR^a)NR^aR^a$, $-OR^a$, $-OC(=O)R^b$, $-OC(=O)NR^aR^a$, $-OC(=O)N(R^a)S(=O)_2R^b$,
 5 $-OC_{2-6}$ alkyl NR^aR^a , $-OC_{2-6}$ alkyl OR^a , $-SR^a$, $-S(=O)R^b$, $-S(=O)_2R^b$, $-S(=O)_2NR^aR^a$, $-S(=O)_2N(R^a)C(=O)R^b$, $-S(=O)_2N(R^a)C(=O)OR^b$, $-S(=O)_2N(R^a)C(=O)NR^aR^a$, $-NR^aR^a$, $-N(R^a)C(=O)R^b$, $-N(R^a)C(=O)OR^b$, $-N(R^a)C(=O)NR^aR^a$, $-N(R^a)C(=NR^a)NR^aR^a$, $-N(R^a)S(=O)_2R^b$, $-N(R^a)S(=O)_2NR^aR^a$, $-NR^aC_{2-6}$ alkyl NR^aR^a and $-NR^aC_{2-6}$ alkyl OR^a .

10 In another embodiment, in conjunction with any of the above or below embodiments, R^3 is phenyl substituted by 1, 2 or 3 substituents selected from C_{1-8} alkyl, C_{1-4} haloalkyl, and halo.

In another embodiment, in conjunction with any of the above or below embodiments, R^3 is phenyl substituted by 1 or 2 chlorine atoms.

15 In another embodiment, in conjunction with any of the above or below embodiments, R^3 is a saturated or unsaturated 5- or 6-membered ring heterocycle containing 1-4 heteroatoms selected from N, O and S, wherein no more than 2 of the heteroatoms are O or S, and the heterocycle is substituted by 0, 1 or 2 oxo groups and is optionally fused with a benzo group, any of which are substituted by 0, 1, 2 or
 20 3 C_{1-8} alkyl, C_{1-4} haloalkyl, halo, cyano, nitro, $-C(=O)R^b$, $-C(=O)OR^b$, $-C(=O)NR^aR^a$, $-C(=NR^a)NR^aR^a$, $-OR^a$, $-OC(=O)R^b$, $-OC(=O)NR^aR^a$, $-OC(=O)N(R^a)S(=O)_2R^b$, $-OC_{2-6}$ alkyl NR^aR^a , $-OC_{2-6}$ alkyl OR^a , $-SR^a$, $-S(=O)R^b$, $-S(=O)_2R^b$, $-S(=O)_2NR^aR^a$, $-S(=O)_2N(R^a)C(=O)R^b$, $-S(=O)_2N(R^a)C(=O)OR^b$, $-S(=O)_2N(R^a)C(=O)NR^aR^a$, $-NR^aR^a$, $-N(R^a)C(=O)R^b$, $-N(R^a)C(=O)OR^b$, $-N(R^a)C(=O)NR^aR^a$,
 25 $-N(R^a)C(=NR^a)NR^aR^a$, $-N(R^a)S(=O)_2R^b$, $-N(R^a)S(=O)_2NR^aR^a$, $-NR^aC_{2-6}$ alkyl NR^aR^a or $-NR^aC_{2-6}$ alkyl OR^a .

In another embodiment, in conjunction with any of the above or below embodiments, R^4 is a saturated or unsaturated 5- or 6-membered ring heterocycle containing 1-4 heteroatoms selected from N, O and S, wherein no more than 2 of the
 30 heteroatoms are O or S, and the heterocycle is substituted by 0, 1 or 2 oxo groups and is optionally fused with a benzo group, any of which are substituted by 0, 1, 2 or 3 substituents selected from C_{1-8} alkyl, C_{1-4} haloalkyl, halo, cyano, nitro, $-C(=O)R^b$,

- C(=O)OR^b, -C(=O)NR^aR^a, -C(=NR^a)NR^aR^a, -OR^a, -OC(=O)R^b, -OC(=O)NR^aR^a,
 -OC(=O)N(R^a)S(=O)₂R^b, -OC₂₋₆alkylNR^aR^a, -OC₂₋₆alkylOR^a, -SR^a, -S(=O)R^b,
 -S(=O)₂R^b, -S(=O)₂NR^aR^a, -S(=O)₂N(R^a)C(=O)R^b, -S(=O)₂N(R^a)C(=O)OR^b,
 -S(=O)₂N(R^a)C(=O)NR^aR^a, -NR^aR^a, -N(R^a)C(=O)R^b, -N(R^a)C(=O)OR^b,
 5 -N(R^a)C(=O)NR^aR^a, -N(R^a)C(=NR^a)NR^aR^a, -N(R^a)S(=O)₂R^b, -N(R^a)S(=O)₂NR^aR^a,
 -NR^aC₂₋₆alkylNR^aR^a and -NR^aC₂₋₆alkylOR^a.

- In another embodiment, in conjunction with any of the above or below
 embodiments, R⁴ is an unsaturated 6-membered ring heterocycle containing 1 or 2
 N atoms, and the heterocycle is substituted by 0, 1, 2 or 3 substituents selected from
 10 C₁₋₈alkyl, C₁₋₄haloalkyl, halo, cyano, nitro, -C(=O)R^b, -C(=O)OR^b, -C(=O)NR^aR^a,
 -C(=NR^a)NR^aR^a, -OR^a, -OC(=O)R^b, -OC(=O)NR^aR^a, -OC(=O)N(R^a)S(=O)₂R^b,
 -OC₂₋₆alkylNR^aR^a, -OC₂₋₆alkylOR^a, -SR^a, -S(=O)R^b, -S(=O)₂R^b, -S(=O)₂NR^aR^a,
 -S(=O)₂N(R^a)C(=O)R^b, -S(=O)₂N(R^a)C(=O)OR^b, -S(=O)₂N(R^a)C(=O)NR^aR^a,
 -NR^aR^a, -N(R^a)C(=O)R^b, -N(R^a)C(=O)OR^b, -N(R^a)C(=O)NR^aR^a,
 15 -N(R^a)C(=NR^a)NR^aR^a, -N(R^a)S(=O)₂R^b, -N(R^a)S(=O)₂NR^aR^a, -NR^aC₂₋₆alkylNR^aR^a
 and -NR^aC₂₋₆alkylOR^a.

In another embodiment, in conjunction with any of the above or below
 embodiments, R⁴ is an unsubstituted, unsaturated 6-membered ring heterocycle
 containing 1 or 2 N atoms.

- 20 In another embodiment, in conjunction with any of the above or below
 embodiments, R⁴ is pyridine.

In another embodiment, in conjunction with any of the above or below
 embodiments, R⁴ is pyrimidine.

- In another embodiment, in conjunction with any of the above or below
 25 embodiments, R⁴ is phenyl or naphthyl, both of which are substituted by 0, 1, 2 or 3
 substituents selected from C₁₋₈alkyl, C₁₋₄haloalkyl, halo, cyano, nitro, -C(=O)R^b,
 -C(=O)OR^b, -C(=O)NR^aR^a, -C(=NR^a)NR^aR^a, -OR^a, -OC(=O)R^b, -OC(=O)NR^aR^a,
 -OC(=O)N(R^a)S(=O)₂R^b, -OC₂₋₆alkylNR^aR^a, -OC₂₋₆alkylOR^a, -SR^a, -S(=O)R^b,
 -S(=O)₂R^b, -S(=O)₂NR^aR^a, -S(=O)₂N(R^a)C(=O)R^b, -S(=O)₂N(R^a)C(=O)OR^b,
 30 -S(=O)₂N(R^a)C(=O)NR^aR^a, -NR^aR^a, -N(R^a)C(=O)R^b, -N(R^a)C(=O)OR^b,
 -N(R^a)C(=O)NR^aR^a, -N(R^a)C(=NR^a)NR^aR^a, -N(R^a)S(=O)₂R^b, -N(R^a)S(=O)₂NR^aR^a,
 -NR^aC₂₋₆alkylNR^aR^a and -NR^aC₂₋₆alkylOR^a.

In another embodiment, in conjunction with any of the above or below embodiments, R^4 is pyridine or pyrimidine, both of which are substituted by 0, 1, 2 or 3 substituents selected from C_{1-8} alkyl, C_{1-4} haloalkyl, halo, cyano, nitro, $-C(=O)R^b$, $-C(=O)OR^b$, $-C(=O)NR^aR^a$, $-C(=NR^a)NR^aR^a$, $-OR^a$, $-OC(=O)R^b$,
 5 $-OC(=O)NR^aR^a$, $-OC(=O)N(R^a)S(=O)_2R^b$, $-OC_{2-6}alkylNR^aR^a$, $-OC_{2-6}alkylOR^a$, $-SR^a$, $-S(=O)R^b$, $-S(=O)_2R^b$, $-S(=O)_2NR^aR^a$, $-S(=O)_2N(R^a)C(=O)R^b$, $-S(=O)_2N(R^a)C(=O)OR^b$, $-S(=O)_2N(R^a)C(=O)NR^aR^a$, $-NR^aR^a$, $-N(R^a)C(=O)R^b$, $-N(R^a)C(=O)OR^b$, $-N(R^a)C(=O)NR^aR^a$, $-N(R^a)C(=NR^a)NR^aR^a$, $-N(R^a)S(=O)_2R^b$, $-N(R^a)S(=O)_2NR^aR^a$, $-NR^aC_{2-6}alkylNR^aR^a$ and $-NR^aC_{2-6}alkylOR^a$.

10 In another embodiment, in conjunction with any of the above or below embodiments, R^4 is not pyridine or phenyl.

Another aspect of the invention relates to a pharmaceutical composition comprising a compound according to any one of the above embodiments and a pharmaceutically acceptable carrier.

15 Another aspect of the invention relates to a method of prophylaxis or treatment of inflammation comprising administering an effective amount of a compound according to any one of the above embodiments.

Another aspect of the invention relates to a method of prophylaxis or treatment of rheumatoid arthritis, Pagets disease, osteoporosis, multiple myeloma,
 20 uveitis, acute or chronic myelogenous leukemia, pancreatic β cell destruction, osteoarthritis, rheumatoid spondylitis, gouty arthritis, inflammatory bowel disease, adult respiratory distress syndrome (ARDS), psoriasis, Crohn's disease, allergic rhinitis, ulcerative colitis, anaphylaxis, contact dermatitis, asthma, muscle degeneration, cachexia, Reiter's syndrome, type I diabetes, type II diabetes, bone
 25 resorption diseases, graft vs. host reaction, Alzheimer's disease, stroke, myocardial infarction, ischemia reperfusion injury, atherosclerosis, brain trauma, multiple sclerosis, cerebral malaria, sepsis, septic shock, toxic shock syndrome, fever, myalgias due to HIV-1, HIV-2, HIV-3, cytomegalovirus (CMV), influenza, adenovirus, the herpes viruses or herpes zoster infection in a mammal comprising
 30 administering an effective amount of a compound according to any one of the above embodiments.

Another aspect of the invention relates to a method of lowering plasma concentrations of either or both TNF- α and IL-1 comprising administering an effective amount of a compound according to any one of the above embodiments.

5 Another aspect of the invention relates to a method of lowering plasma concentrations of either or both IL-6 and IL-8 comprising administering an effective amount of a compound according to any one of the above embodiments.

Another aspect of the invention relates to a method of prophylaxis or treatment of diabetes disease in a mammal comprising administering an effective amount of a compound according to any one of the above embodiments to produce a
10 glucagon antagonist effect.

Another aspect of the invention relates to a method of prophylaxis or treatment of a pain disorder in a mammal comprising administering an effective amount of a compound according to any one of the above embodiments.

15 Another aspect of the invention relates to a method of decreasing prostaglandins production in a mammal comprising administering an effective amount of a compound according to any one of the above embodiments.

Another aspect of the invention relates to a method of decreasing cyclooxygenase enzyme activity in a mammal comprising administering an effective amount of a compound according to any one of the above embodiments. In another
20 embodiment, the cyclooxygenase enzyme is COX-2.

Another aspect of the invention relates to a method of decreasing cyclooxygenase enzyme activity in a mammal comprising administering an effective amount of the above pharmaceutical composition. In another embodiment the cyclooxygenase enzyme is COX-2.

25 Another aspect of the invention relates to the manufacture of a medicament comprising a compound according to any one of the above embodiments.

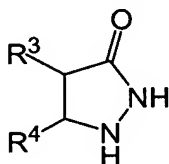
Another aspect of the invention relates to the manufacture of a medicament for the treatment of inflammation comprising administering an effective amount of a compound according to any one of the above embodiments.

30 Another aspect of the invention relates to the manufacture of a medicament for the treatment of rheumatoid arthritis, Pagets disease, osteoporosis, multiple myeloma, uveitis, acute or chronic myelogenous leukemia, pancreatic β cell

destruction, osteoarthritis, rheumatoid spondylitis, gouty arthritis, inflammatory bowel disease, adult respiratory distress syndrome (ARDS), psoriasis, Crohn's disease, allergic rhinitis, ulcerative colitis, anaphylaxis, contact dermatitis, asthma, muscle degeneration, cachexia, Reiter's syndrome, type I diabetes, type II diabetes, bone resorption diseases, graft vs. host reaction, Alzheimer's disease, stroke, myocardial infarction, ischemia reperfusion injury, atherosclerosis, brain trauma, multiple sclerosis, cerebral malaria, sepsis, septic shock, toxic shock syndrome, fever, myalgias due to HIV-1, HIV-2, HIV-3, cytomegalovirus (CMV), influenza, adenovirus, the herpes viruses or herpes zoster infection in a mammal comprising administering an effective amount of a compound according to any one of the above embodiments.

Another aspect of the invention relates to a method of making a compound as described herein, comprising the steps of:

- reacting $R^3\text{-CO}_2\text{H}$ with $R^4\text{-C(=O)H}$ in the presence of trialkylamine and acetic anhydride;
- protecting the resulting acid with a protecting group; and
- reacting the protected acid with hydrazine to form



The compounds of this invention may have in general several asymmetric centers and are typically depicted in the form of racemic mixtures. This invention is intended to encompass racemic mixtures, partially racemic mixtures and separate enantiomers and diastereomers.

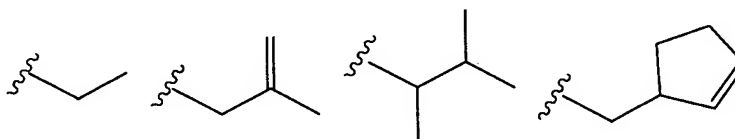
The specification and claims contain listing of species using the language "selected from . . . and . . ." and "is . . . or . . ." (sometimes referred to as Markush groups). When this language is used in this application, unless otherwise stated it is meant to include the group as a whole, or any single members thereof, or any subgroups thereof. The use of this language is merely for shorthand purposes and is not meant in any way to limit the removal of individual elements or subgroups from the genus.

Unless otherwise specified, the following definitions apply to terms found in the specification and claims:

"Aryl" means a phenyl or naphthyl radical, wherein the phenyl may be fused with a C₃₋₄cycloalkyl bridge.

- 5 "Benzo group", alone or in combination, means the divalent radical C₄H₄=, one representation of which is -CH=CH-CH=CH-, that when vicinally attached to another ring forms a benzene-like ring--for example tetrahydronaphthylene, indole and the like.

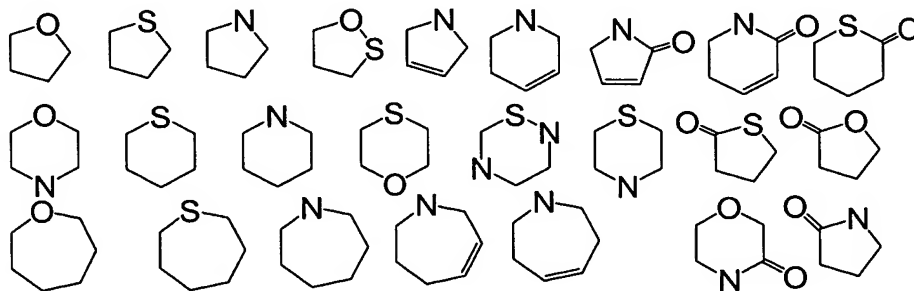
- "C_{α-β}alkyl" means an alkyl group comprising from α to β carbon atoms in a
 10 branched, cyclical or linear relationship or any combination of the three. The alkyl groups described in this section may also contain double or triple bonds. Examples of C₁₋₈alkyl include, but are not limited to the following:

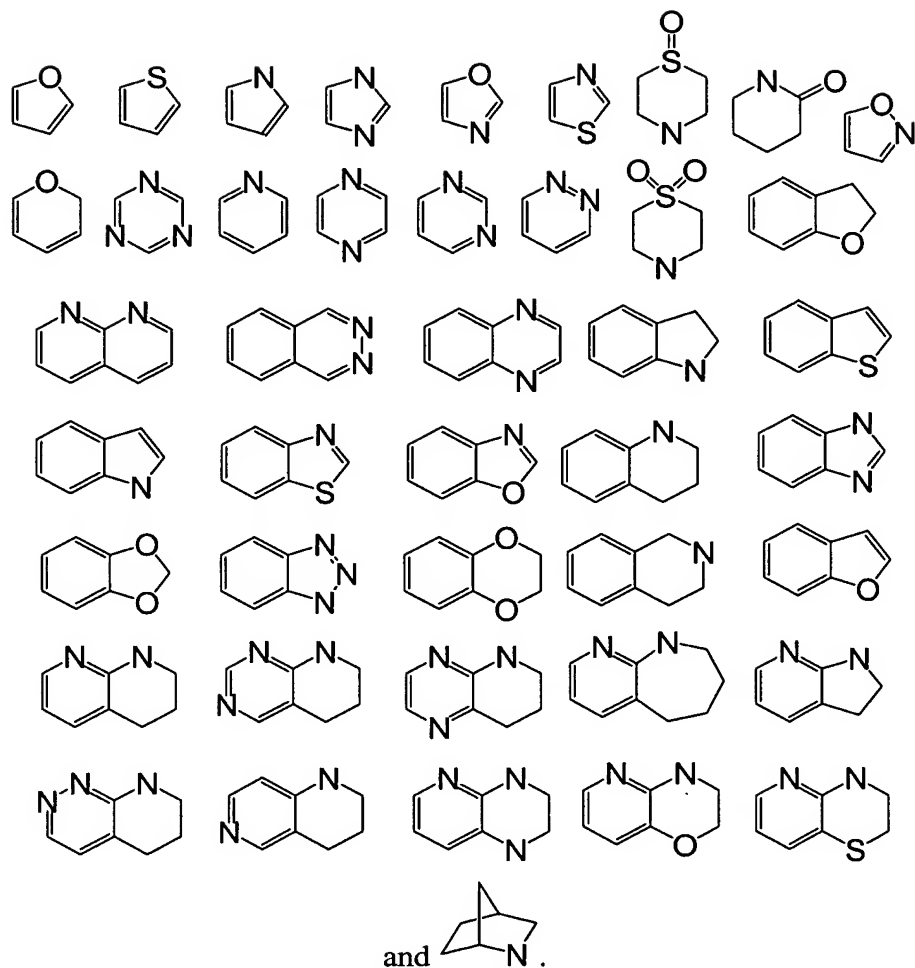


"Halogen" and "halo" mean a halogen atoms selected from F, Cl, Br and I.

- 15 "C_{α-β}haloalkyl" means an alkyl group, as described above, wherein any number--at least one--of the hydrogen atoms attached to the alkyl chain are replaced by F, Cl, Br or I.

- "Heterocycle" means a ring comprising at least one carbon atom and at least one other atom selected from N, O and S. Examples of heterocycles that may be found
 20 in the claims include, but are not limited to, the following:





- 5 "Pharmaceutically-acceptable salt" means a salt prepared by conventional means, and are well known by those skilled in the art. The "pharmacologically acceptable salts" include basic salts of inorganic and organic acids, including but not limited to hydrochloric acid, hydrobromic acid, sulfuric acid, phosphoric acid, methanesulphonic acid, ethanesulfonic acid, malic acid, acetic acid, oxalic acid,
- 10 tartaric acid, citric acid, lactic acid, fumaric acid, succinic acid, maleic acid, salicylic acid, benzoic acid, phenylacetic acid, mandelic acid and the like. When compounds of the invention include an acidic function such as a carboxy group, then suitable pharmaceutically acceptable cation pairs for the carboxy group are well known to those skilled in the art and include alkaline, alkaline earth, ammonium, quaternary
- 15 ammonium cations and the like. For additional examples of "pharmacologically acceptable salts," *see infra* and Berge et al., J. Pharm. Sci. 66:1 (1977).

"Leaving group" generally refers to groups readily displaceable by a nucleophile, such as an amine, a thiol or an alcohol nucleophile. Such leaving groups are well known in the art. Examples of such leaving groups include, but are not limited to, N-hydroxysuccinimide, N-hydroxybenzotriazole, halides, triflates, tosylates and the like. Preferred leaving groups are indicated herein where appropriate.

"Protecting group" generally refers to groups well known in the art which are used to prevent selected reactive groups, such as carboxy, amino, hydroxy, mercapto and the like, from undergoing undesired reactions, such as nucleophilic, electrophilic, oxidation, reduction and the like. Preferred protecting groups are indicated herein

where appropriate. Examples of amino protecting groups include, but are not limited to, aralkyl, substituted aralkyl, cycloalkenylalkyl and substituted cycloalkenyl alkyl, allyl, substituted allyl, acyl, alkoxycarbonyl, aralkoxycarbonyl, silyl and the like.

Examples of aralkyl include, but are not limited to, benzyl, ortho-methylbenzyl, trityl and benzhydryl, which can be optionally substituted with halogen, alkyl, alkoxy,

hydroxy, nitro, acylamino, acyl and the like, and salts, such as phosphonium and ammonium salts. Examples of aryl groups include phenyl, naphthyl, indanyl, anthracenyl, 9-(9-phenylfluorenyl), phenanthrenyl, durenyl and the like. Examples of cycloalkenylalkyl or substituted cycloalkenylalkyl radicals, preferably have 6-10 carbon atoms, include, but are not limited to, cyclohexenyl methyl and the like.

Suitable acyl, alkoxycarbonyl and aralkoxycarbonyl groups include benzyloxycarbonyl, t-butoxycarbonyl, iso-butoxycarbonyl, benzoyl, substituted benzoyl, butyryl, acetyl, tri-fluoroacetyl, tri-chloro acetyl, phthaloyl and the like. A mixture of protecting groups can be used to protect the same amino group, such as a primary amino group can be protected by both an aralkyl group and an aralkoxycarbonyl group. Amino protecting groups can also form a heterocyclic ring with the nitrogen to which they are attached, for example, 1,2-bis(methylene)benzene, phthalimidyl, succinimidyl, maleimidyl and the like and where these heterocyclic groups can further include adjoining aryl and cycloalkyl rings. In addition, the heterocyclic groups can be mono-, di- or tri-substituted, such as nitrophthalimidyl.

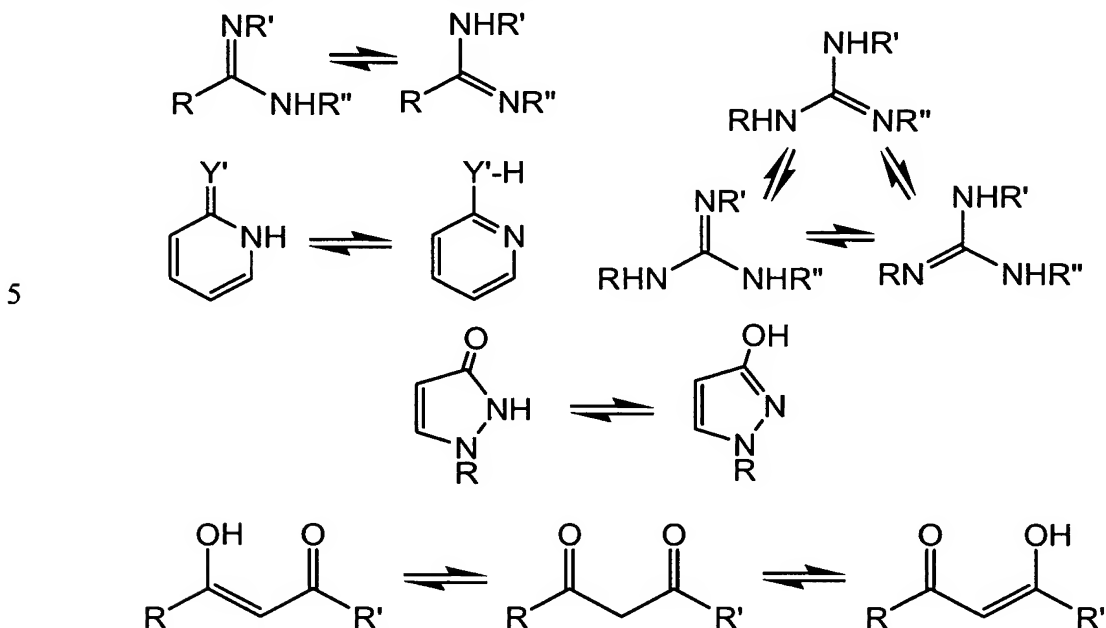
Amino groups may also be protected against undesired reactions, such as oxidation, through the formation of an addition salt, such as hydrochloride, toluenesulfonic acid, trifluoroacetic acid and the like. Many of the amino protecting groups are also

suitable for protecting carboxy, hydroxy and mercapto groups. For example, aralkyl groups. Alkyl groups are also suitable groups for protecting hydroxy and mercapto groups, such as tert-butyl.

Silyl protecting groups are silicon atoms optionally substituted by one or
5 more alkyl, aryl and aralkyl groups. Suitable silyl protecting groups include, but are not limited to, trimethylsilyl, triethylsilyl, tri-isopropylsilyl, tert-butyldimethylsilyl, dimethylphenylsilyl, 1,2-bis(dimethylsilyl)benzene, 1,2-bis(dimethylsilyl)ethane and diphenylmethylsilyl. Silylation of an amino groups provide mono- or di-silylamino groups. Silylation of aminoalcohol compounds can lead to a N,N,O-tri-silyl
10 derivative. Removal of the silyl function from a silyl ether function is readily accomplished by treatment with, for example, a metal hydroxide or ammonium fluoride reagent, either as a discrete reaction step or in situ during a reaction with the alcohol group. Suitable silylating agents are, for example, trimethylsilyl chloride, tert-butyl-dimethylsilyl chloride, phenyldimethylsilyl chloride,
15 diphenylmethyl silyl chloride or their combination products with imidazole or DMF. Methods for silylation of amines and removal of silyl protecting groups are well known to those skilled in the art. Methods of preparation of these amine derivatives from corresponding amino acids, amino acid amides or amino acid esters are also well known to those skilled in the art of organic chemistry including amino
20 acid/amino acid ester or aminoalcohol chemistry.

Protecting groups are removed under conditions which will not affect the remaining portion of the molecule. These methods are well known in the art and include acid hydrolysis, hydrogenolysis and the like. A preferred method involves removal of a protecting group, such as removal of a benzyloxycarbonyl group by
25 hydrogenolysis utilizing palladium on carbon in a suitable solvent system such as an alcohol, acetic acid, and the like or mixtures thereof. A t-butoxycarbonyl protecting group can be removed utilizing an inorganic or organic acid, such as HCl or trifluoroacetic acid, in a suitable solvent system, such as dioxane or methylene chloride. The resulting amino salt can readily be neutralized to yield the free amine.
30 Carboxy protecting group, such as methyl, ethyl, benzyl, tert-butyl, 4-methoxyphenylmethyl and the like, can be removed under hydrolysis and hydrogenolysis conditions well known to those skilled in the art.

It should be noted that compounds of the invention may contain groups that may exist in tautomeric forms, such as cyclic and acyclic amidine and guanidine groups, heteroatom substituted heteroaryl groups ($Y' = O, S, NR$), and the like, which are illustrated in the following examples:



and though one form is named, described, displayed and/or claimed herein, all the tautomeric forms are intended to be inherently included in such name, description, display and/or claim.

10 Prodrugs of the compounds of this invention are also contemplated by this invention. A prodrug is an active or inactive compound that is modified chemically through in vivo physiological action, such as hydrolysis, metabolism and the like, into a compound of this invention following administration of the prodrug to a patient. The suitability and techniques involved in making and using prodrugs are

15 well known by those skilled in the art. For a general discussion of prodrugs involving esters see Svensson and Tunek Drug Metabolism Reviews 165 (1988) and Bundgaard Design of Prodrugs, Elsevier (1985). Examples of a masked carboxylate anion include a variety of esters, such as alkyl (for example, methyl, ethyl), cycloalkyl (for example, cyclohexyl), aralkyl (for example, benzyl, p-

20 methoxybenzyl), and alkylcarbonyloxyalkyl (for example, pivaloyloxymethyl). Amines have been masked as arylcarbonyloxymethyl substituted derivatives which are cleaved by esterases in vivo releasing the free drug and formaldehyde

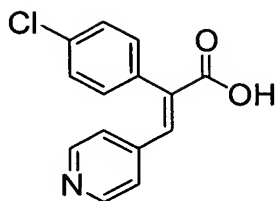
(Bundgaard J. Med. Chem. 2503 (1989)). Also, drugs containing an acidic NH group, such as imidazole, imide, indole and the like, have been masked with N-acyloxymethyl groups (Bundgaard Design of Prodrugs, Elsevier (1985)). Hydroxy groups have been masked as esters and ethers. EP 039,051 (Sloan and Little,
5 4/11/81) discloses Mannich-base hydroxamic acid prodrugs, their preparation and use.

"Cytokine" means a secreted protein that affects the functions of other cells, particularly as it relates to the modulation of interactions between cells of the immune system or cells involved in the inflammatory response. Examples of
10 cytokines include but are not limited to interleukin 1 (IL-1), preferably IL-1 β , interleukin 6 (IL-6), interleukin 8 (IL-8) and TNF, preferably TNF- α (tumor necrosis factor- α).

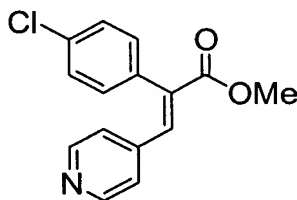
"TNF, IL-1, IL-6, and/or IL-8 mediated disease or disease state" means all disease states wherein TNF, IL-1, IL-6, and/or IL-8 plays a role, either directly as TNF, IL-
15 1, IL-6, and/or IL-8 itself, or by TNF, IL-1, IL-6, and/or IL-8 inducing another cytokine to be released. For example, a disease state in which IL-1 plays a major role, but in which the production of or action of IL-1 is a result of TNF, would be considered mediated by TNF.

Compounds according to the invention can be synthesized according to one
20 or more of the following methods. It should be noted that the general procedures are shown as it relates to preparation of compounds having unspecified stereochemistry. However, such procedures are generally applicable to those compounds of a specific stereochemistry, e.g., where the stereochemistry about a group is (S) or (R). In addition, the compounds having one stereochemistry (e.g., (R)) can often be utilized
25 to produce those having opposite stereochemistry (i.e., (S)) using well-known methods, for example, by inversion.

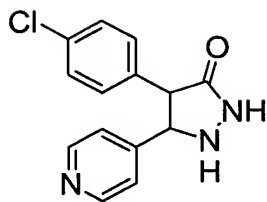
The following Examples are presented for illustrative purposes only and are not intended, nor should they be construed, as limiting the invention in any manner. Those skilled in the art will appreciate that modifications and variations of the
30 compounds disclosed herein can be made without violating the spirit or scope of the present invention.

EXAMPLES**Example 1****2-(4-Chloro-phenyl)-3-pyridin-4-yl-acrylic acid**

- 5 To mixture of (4-Chloro-phenyl)-acetic acid 9.78 g and pyridine-4-carbaldehyde 6.14 g was added acetic anhydride 20 mL and triethyl amine 20 mL. The reaction mixture was heated to 120 °C for 24 hours and cooled down to room temperature. Water and ethyl acetate were added. The solids formed was filtered, washed with water and ethyl acetate and dried to afford the title compound as a light yellow solid.
- 10 MS (ES⁺): 260 (M+H)⁺.

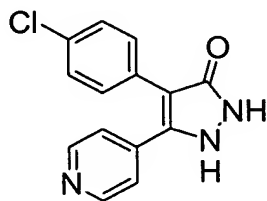
Example 2**2-(4-Chloro-phenyl)-3-pyridin-4-yl-acrylic acid methyl ester**

- The solution of 2-(4-Chloro-phenyl)-3-pyridin-4-yl-acrylic acid 3.5 g in thionyl chloride 25 mL was heated to 70 °C for five hours. The reaction was cooled to 0 °C,
- 15 then was added methanol 50 mL. After warm to room temperature over one hour, all the solvent removed under reduced pressure. Work up using ethyl acetate and sodium bicarbonate give the light yellow solid. MS (ES⁺): 274 (M+H)⁺.

Example 3

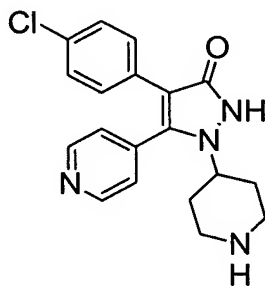
4-(4-Chloro-phenyl)-5-pyridin-4-yl-pyrazolidin-3-one

To a solution of 2-(4-Chloro-phenyl)-3-pyridin-4-yl-acrylic acid methyl ester 2.02 g
 5 in ethyl alcohol 25 mL was added hydrazine 2.37 g. The reaction was heated to 50 °C for 135 minutes. All solvent was removed after under reduced pressure to afford the title compound as light yellow solid. MS (ES⁺): 274 (M+H)⁺.

Example 4

10 4-(4-Chloro-phenyl)-5-pyridin-4-yl-1,2-dihydro-pyrazol-3-one

To solution of 4-(4-Chloro-phenyl)-5-pyridin-4-yl-pyrazolidin-3-one 20 mg in ethyl
 alcohol 5 mL was added Pd/C 1 mg. The reaction was heated to 50 °C for 48 hours,
 cooled to room temperature, filtered and concentrated to a solid. Purification by
 TLC plate followed by crystallization from chloroform and methanol give the title
 15 compound as an off-white solid. MS (ES⁺): 272 (M+H)⁺.

Example 5

4-(4-Chloro-phenyl)-1-piperidin-4-yl-5-pyridin-4-yl-1,2-dihydro-pyrazol-3-one

Step A: 4-[4-(4-Chloro-phenyl)-3-oxo-5-pyridin-4-yl-pyrazolidin-1-yl]-piperidine-1-
 20 carboxylic acid tert-butyl ester

To a solution of 4-(4-Chloro-phenyl)-5-pyridin-4-yl-pyrazolidin-3-one 0.86 g and 4-Oxo-piperidine-1-carboxylic acid tert-butyl ester 0.75 g in chloroform 35 mL was added sodium triacetoxy boron hydride 0.75 g at 0 °C, the reaction was warmed up to room temperature over two hours before heated to 50 °C for another two hours.

- 5 The reaction was quenched with water 25 mL at 0 °C, and the organic layer was collected, filtered, dried over MgSO₄, concentrated to a yellow solid 0.42 g for being directly used for next step. MS (ES⁺): 457 (M+H)⁺.

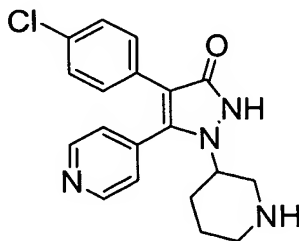
Step B: 4-[4-(4-Chloro-phenyl)-3-oxo-5-pyridin-4-yl-2,3-dihydro-pyrazol-1-yl]-piperidine-1-carboxylic acid tert-butyl ester

- 10 To a solution of 4-[4-(4-Chloro-phenyl)-3-oxo-5-pyridin-4-yl-pyrazolidin-1-yl]-piperidine-1-carboxylic acid tert-butyl ester 0.42 g in ethanol 25 mL was added palladium on carbon 0.02 g. the reaction was heated to 50 °C for 48 hours. The reaction was cooled to room temperature, filtered and concentrated. Purification by flash chromatography gave the title compound as colorless oil. MS (ES⁺): 455 (M+H)⁺; (ES⁻): 453 (M-H).

Step C: 4-(4-Chloro-phenyl)-1-piperidin-4-yl-5-pyridin-4-yl-1,2-dihydro-pyrazol-3-one

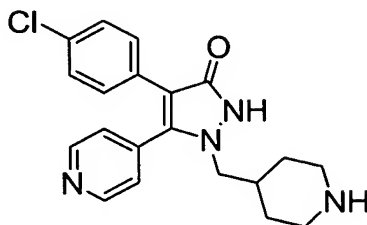
- To a solution of 4-[4-(4-Chloro-phenyl)-3-oxo-5-pyridin-4-yl-2,3-dihydro-pyrazol-1-yl]-piperidine-1-carboxylic acid tert-butyl ester 200 mg in flask was added HCl in ether and dioxane. After 2.25 hours, the solvent was evaporated under reduced pressure to afford the title compound as yellow oil. MS (ES⁺): 355 (M+H)⁺; (ES⁻): 353 (M-H).

Example 6



- 25 4-(4-Chloro-phenyl)-1-piperidin-3-yl-5-pyridin-4-yl-1,2-dihydro-pyrazol-3-one
The title compound was analogously synthesized by the method described in **example 5** from 3-Oxo-piperidine-1-carboxylic acid tert-butyl ester. This

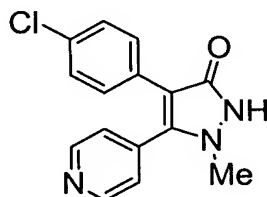
compound was obtained as yellow solid. MS (ES⁺): 355 (M+H)⁺; (ES⁻): 353 (M-H)⁻.

Example 7

- 5 4-(4-Chloro-phenyl)-1-piperidin-4-ylmethyl-5-pyridin-4-yl-1,2-dihydro-pyrazol-3-one

The title compound was analogously synthesized by the method described in **example 5** from 4-Formyl-piperidine-1-carboxylic acid tert-butyl ester. This compound was obtained as yellow solid. MS (ES⁺): 369 (M+H)⁺; (ES⁻): 367 (M-H)⁻.

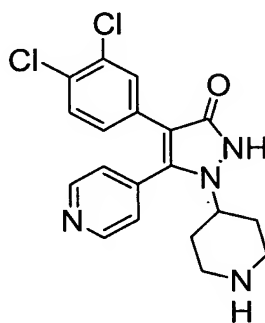
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Example 8

4-(4-Chloro-phenyl)-1-methyl-5-pyridin-4-yl-1,2-dihydro-pyrazol-3-one

The title compound was analogously synthesized by the method described in

- 15 **example 5** from formaldehyde. This compound was obtained as yellow solid. MS (ES⁺): 286 (M+H)⁺; (ES⁻): 284 (M-H)⁻.

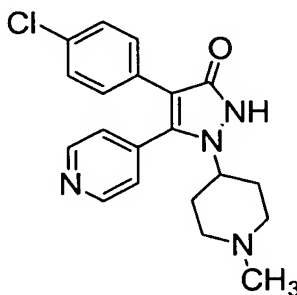
Example 9

4-(3,4-Dichlorophenyl)-1-piperidin-4-yl-5-pyridin-4-yl-1,2-dihydro-pyrazol-3-one

The title compound was synthesized analogously by the method described in

Example 5. 3,4-Dichloro- phenylacetic acid, instead of 4-chlorophenylacetic acid, was used. MS (ES⁺): 389.0 (M+H)⁺; (ES⁻): 387.4 (M-H)⁻.

Example 10



4-(4-Chlorophenyl)-1-(1-methyl-piperidin-4-yl)-5-pyridin-4-yl-1,2-dihydro-pyrazol-3-one

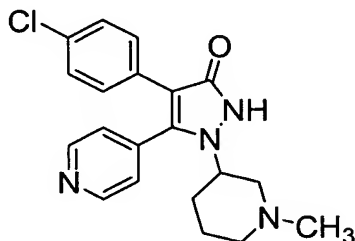
A solution of 4-(4-chlorophenyl)-1-piperidin-4-yl-5-pyridin-4-yl-1,2-dihydro-pyrazol-3-one (0.12 g, 0.34 mmol) in anhydrous methanol (3 mL) was cooled to 0

°C in an ice-water bath and added formaldehyde (3 mL; 37 wt. % in water), followed by sodium borohydride (0.039 g, 1 mmol). After stirring for 1 hour, the reaction was quenched with water and diluted with methylene chloride. The organic layer was washed with brine, dried (Na₂SO₄), and concentrated in vacuo.

The title compound was isolated as a yellow amorphous solid by preparative HPLC.

MS (ES⁺): 369.2 (M+H)⁺; (ES⁻): 367.1 (M-H)⁻.

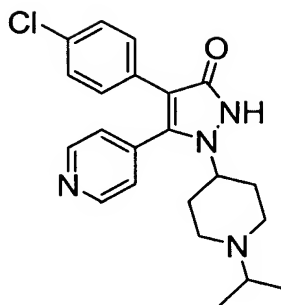
Example 11



4-(4-Chlorophenyl)-1-(1-methyl-piperidin-3-yl)-5-pyridin-4-yl-1,2-dihydro-pyrazol-3-one

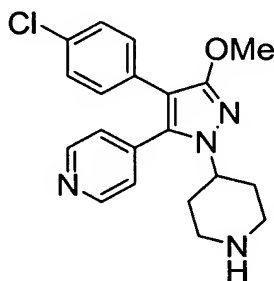
The title compound was synthesized analogously by the method described in

Example 10. 4-(4-Chlorophenyl)-1-piperidin-3-yl-5-pyridin-4-yl-1,2-dihydro-pyrazol-3-one, instead of 4-(4-chlorophenyl)-1-piperidin-4-yl-5-pyridin-4-yl-1,2-dihydro-pyrazol-3-one, was used. MS (ES⁺): 369.2 (M+H)⁺; (ES⁻): 367.1 (M-H)⁻.

Example 12

4-(4-Chlorophenyl)-1-(1-isopropyl-piperidin-4-yl)-5-pyridin-4-yl-1,2-dihydro-pyrazol-3-one

- 5 The title compound was synthesized analogously by the method described in **Example 10**. Acetone, instead of formaldehyde, was used. MS (ES⁺): 397.3 (M+H)⁺; (ES⁻): 395.2 (M-H)⁻.

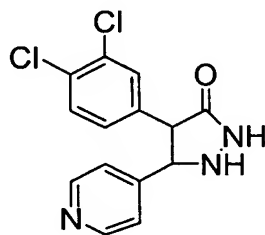
Example 13

- 10 4-[4-(4-Chlorophenyl)-3-methoxy-5-pyridin-4-yl-pyrazol-1-yl]-piperidine
 Step A: 4-[4-(4-Chlorophenyl)-3-methoxy-5-pyridin-4-yl-pyrazol-1-yl]-piperidine-1-carboxylic acid *tert*-butyl ester
 A solution of 4-[4-(4-chlorophenyl)-3-oxo-5-pyridin-4-yl-2,3-dihydro-pyrazol-1-yl]-piperidine-1-carboxylic acid *tert*-butyl ester (0.135g, 0.3 mmol) in dry DMF (2 mL)
 15 was cooled to 0 °C and added lithium hydride (0.0035 g, 0.45 mmol). After stirring for 5 minutes, iodomethane (0.037 mL, 0.6 mmol) was added and the reaction was allowed to gradually warmed to room temperature and stirred for 20 hours. The reaction was diluted with ethyl acetate and washed with water; the aqueous layer was back-washed with ethyl acetate. The combined organic layer was washed with
 20 brine, dried (Na₂SO₄), and concentrated in vacuo. Flash chromatography with 1%, 3%, and 5% methanol/methylene chloride afforded 18.8 mg (13 %) of a monomethylated product. MS (ES⁺): 469.2 (M+H)⁺.

Step B: 4-[4-(4-Chlorophenyl)-3-methoxy-5-pyridin-4-yl-pyrazol-1-yl]-piperidine

Following the procedure of Step C in **Example 5**, the title compound was isolated as a yellow amorphous solid. MS (ES⁺): 369.2 (M+H)⁺.

Example 14



4-(3,4-Dichloro-phenyl)-5-pyridin-4-yl-pyrazolidin-3-one

Step A: 3-(3,4-Dichloro-phenyl)-2-oxo-4-pyridin-4-yl-but-3-enoic acid

To mixture of (3,4-diChloro-phenyl)-acetic acid 10.3 g and pyridine-4-carbaldehyde 5.25 mL was added acetic anhydride 6 mL and pyridine 6 mL. The reaction mixture was heated to 120 °C for 2 hours and cooled down to room temperature. Water and ethyl acetate were added. The solid formed was filtered, washed with water and ethyl acetate and dried to afford the title compound 10.2 g as a light yellow solid.

MS

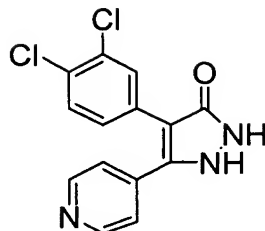
(ES⁺): 292 (M+H)⁺.

Step B: 3-(3,4-Dichloro-phenyl)-2-oxo-4-pyridin-4-yl-but-3-enoic acid ethyl ester

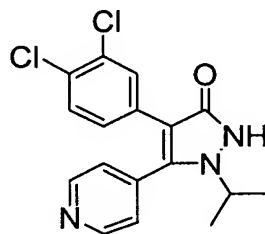
The solution of 3-(3,4-Dichloro-phenyl)-2-oxo-4-pyridin-4-yl-but-3-enoic acid 9.6 g in thionyl chloride 20 mL was heated to 80 °C for 1 hours. Vacuumed down the excess thionyl chloride, cooled to 0 °C, then added methanol 50 mL. After warmed to room temperature over one hour and 60 °C for 1 h, all the solvent removed under reduced pressure. Work up using ethyl acetate and sodium bicarbonate give the title compound 11.43 g as white solid. MS (ES⁺): 322 (M+H)⁺.

Step C: 4-(3,4-Dichloro-phenyl)-5-pyridin-4-yl-pyrazolidin-3-one

To a solution of 3-(3,4-Dichloro-phenyl)-2-oxo-4-pyridin-4-yl-but-3-enoic acid ethyl ester 11.4 g in ethyl alcohol 50 mL was added hydrazine 1.68 mL. The reaction was heated to 50 °C for 15 hours. All solvent was removed after under reduced pressure to afford the title compound 10.8 g as light yellow solid. MS (ES⁺): 308 (M+H)⁺.

Example 15**4-(3,4-Dichloro-phenyl)-5-pyridin-4-yl-1,2-dihydro-pyrazol-3-one**

The solution of 4-(3,4-Dichloro-phenyl)-5-pyridin-4-yl-pyrazolidin-3-one 0.30 g, 20
 5 mg Pd/C and 20 mL methanol was stirred at room temperature. Air was bubbled
 through for 2 hours. Filtered off the Pd/C catalyst, vacuumed down all solvent. The
 title compound was obtained as white solid 0.29 g. MS (ES⁺): 306 (M+H)⁺.

Example 16**10 4-(3,4-Dichloro-phenyl)-1-isopropyl-5-pyridin-4-yl-1,2-dihydro-pyrazol-3-one****Step A: 4-(3,4-Dichloro-phenyl)-1-isopropyl-5-pyridin-4-yl-pyrazolidin-3-one**

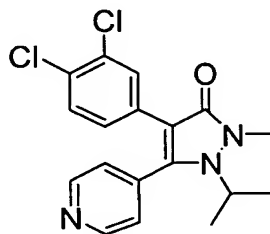
To a solution of 4-(3,4-Dichloro-phenyl)-5-pyridin-4-yl-pyrazolidin-3-one 0.30 g
 and 0.1 mL acetone in chloroform 20 mL was added NaBH₃(CN) 0.1 g at 0 °C, the
 reaction was warmed 50 °C and stirred for 1 hour. The reaction was quenched with
 15 sat. NaHCO₃ 25 mL at 0 °C. The reaction mixture was extracted with
 dichloromethane, 3 x 50 mL. The combined organic phase was washed with brine,
 dried over anhydrous Na₂SO₄. After purification by flash chromatography, the title
 compound was obtained as yellow solid 0.26 g. MS (ES⁺): 350 (M+H)⁺.

20 Step B: 4-(3,4-Dichloro-phenyl)-1-isopropyl-5-pyridin-4-yl-1,2-dihydro-pyrazol-3-one

The solution of 4-(3,4-Dichloro-phenyl)-1-isopropyl-5-pyridin-4-yl-pyrazolidin-3-
 one 90 mg in dichloromethane 10 mL was treated with phenyltriethylammonium
 tribromide 0.1 g at room temperature, The reaction mixture was stirred at room
 temperature for 15 h. The reaction was quenched with sat. Na₂SO₃ 25 mL at 0 °C.

The reaction mixture was extracted with dichloromethane, 3 x 50 mL. The combined organic phase was washed with brine, dried over anhydrous Na₂SO₄. After purification by flash chromatography, the title compound was obtained as light yellow solid 45 mg. MS (ES⁺): 348 (M+H)⁺.

5 **Example 17**



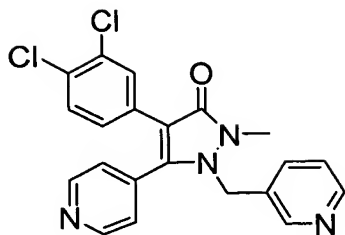
4-(3,4-Dichloro-phenyl)-1-isopropyl-2-methyl-5-pyridin-4-yl-1,2-dihydro-pyrazol-3-one

10 Step A: 4-(3,4-Dichloro-phenyl)-1-isopropyl-2-methyl-5-pyridin-4-yl-pyrazolidin-3-one

In a 100 mL round bottom flask, was added 0.1 g 4-(3,4-Dichloro-phenyl)-1-isopropyl-5-pyridin-4-yl-pyrazolidin-3-one and 10 mL THF, stirred at 0 °C under nitrogen. 0.43 mL 1 M LHMDs in THF was added drop wise. After stirred at 0 °C for 30 min 0.026 mL iodomethane was added drop wise. The resulted mixture was stirred from 0 °C to rt for 2 hours, quenched with 20 mL sat. NH₄Cl, extracted with dichloromethane 3 x 25 mL. The organic phase was dried over anhydrous Na₂SO₄. After purification by flash chromatography, obtained the title compound 80 mg as light solid. MS (ES⁺): 364 (M+H)⁺.

15 Step B: 4-(3,4-Dichloro-phenyl)-1-isopropyl-2-methyl-5-pyridin-4-yl-1,2-dihydro-pyrazol-3-one

20 The solution of 4-(3,4-Dichloro-phenyl)-1-isopropyl-2-methyl-5-pyridin-4-yl-pyrazolidin-3-one 40 mg in dichloromethane 10 mL was treated with phenyltriethylammonium tribromide 0.1 g at room temperature, The reaction mixture was stirred at room temperature for 15 h. The reaction was quenched with sat. Na₂SO₃ 25 mL at 0 °C. The reaction mixture was extracted with dichloromethane, 3 x 25 mL. The combined organic phase was washed with brine, dried over anhydrous Na₂SO₄. After purification by flash chromatography, the title compound was obtained as light yellow solid 20 mg. MS (ES⁺): 362 (M+H)⁺.

Example 18

4-(3,4-Dichloro-phenyl)-2-methyl-5-pyridin-4-yl-1-pyridin-3-ylmethyl-1,2-dihydro-pyrazol-3-one

- 5 Step A: 4-(3,4-Dichloro-phenyl)-5-pyridin-4-yl-1-pyridin-3-ylmethyl-pyrazolidin-3-one

To a solution of 4-(3,4-Dichloro-phenyl)-5-pyridin-4-yl-pyrazolidin-3-one 0.30 g and 0.12 mL 3-pyridinecarboxaldehyde in chloroform 20 mL was added

NaBH₃(CN) 0.1 g at 0 °C, the reaction was warmed 50 °C and stirred for 1 hour.

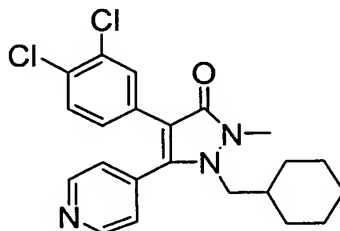
- 10 The reaction was quenched with sat. NaHCO₃ 25 mL at 0 °C. The reaction mixture was extracted with dichloromethane, 3 x 50 mL. The combined organic phase was washed with brine, dried over anhydrous Na₂SO₄. After purification by flash chromatography, the title compound was obtained as yellow solid 0.28 g. MS (ES⁺): 399 (M+H)⁺.

- 15 Step B: 4-(3,4-Dichloro-phenyl)-2-methyl-5-pyridin-4-yl-1-pyridin-3-ylmethyl-pyrazolidin-3-one and 4-(3,4-Dichloro-phenyl)-2-methyl-5-pyridin-4-yl-1-pyridin-3-ylmethyl-1,2-dihydro-pyrazol-3-one

In a 100 mL round bottom flask, was added 70 mg 4-(3,4-Dichloro-phenyl)-5-pyridin-4-yl-1-pyridin-3-ylmethyl-pyrazolidin-3-one and 10 mL THF, stirred at 0 °C

- 20 under nitrogen. 0.26 mL 1 M LHMDS in THF was added drop wise. After stirred at 0 °C for 30 min. 0.017 mL iodomethane was added drop wise. The resulted mixture was stirred from 0 °C to rt for 2 hour, quenched with 20 mL sat. NH₄Cl, extracted with dichloromethane 3 x 25 mL. The crude MS showed the desired 4-(3,4-Dichloro-phenyl)-2-methyl-5-pyridin-4-yl-1-pyridin-3-ylmethyl-pyrazolidin-3-one
- 25 was there, MS (ES⁺): 413 (M+H)⁺. The organic phase was dried over anhydrous Na₂SO₄. After purification by flash chromatography, the 4-(3,4-Dichloro-phenyl)-5-pyridin-4-yl-1-pyridin-3-ylmethyl-pyrazolidin-3-one was oxidized on column and

obtained the 4-(3,4-Dichloro-phenyl)-2-methyl-5-pyridin-4-yl-1-pyridin-3-ylmethyl-1,2-dihydro-pyrazol-3-one 12 mg as light yellow solid. MS (ES⁺): 411 (M+H)⁺.

Example 19

5 1-Cyclohexylmethyl-4-(3,4-dichloro-phenyl)-2-methyl-5-pyridin-4-yl-1,2-dihydro-pyrazol-3-one

Step A: 1-Cyclohexylmethyl-4-(3,4-dichloro-phenyl)-5-pyridin-4-yl-pyrazolidin-3-one

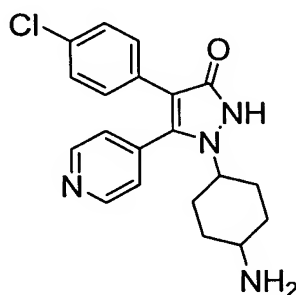
To a solution of 4-(3,4-Dichloro-phenyl)-5-pyridin-4-yl-pyrazolidin-3-one 0.30 g
10 and 0.2 mL cyclohexanecarbaldehyde in chloroform 20 mL was added NaBH₃(CN) 0.1 g at 0 °C, the reaction was warmed 50 °C and stirred for 1 hour. The reaction was quenched with sat.NaHCO₃ 25 mL at 0 °C. The reaction mixture was extracted with dichloromethane, 3 x 50 mL. The combined organic phase was washed with brine, dried over anhydrous Na₂SO₄. After purification by flash chromatography, the
15 title compound was obtained as yellow solid 0.4 g. MS (ES⁺): 404 (M+H)⁺.

Step B: 1-Cyclohexylmethyl-4-(3,4-dichloro-phenyl)-2-methyl-5-pyridin-4-yl-pyrazolidin-3-one

In a 100 mL round bottom flask, was added 0.4 g 1-Cyclohexylmethyl-4-(3,4-dichloro-phenyl)-5-pyridin-4-yl-pyrazolidin-3-one and 20 mL THF, stirred at 0 °C
20 under nitrogen. 1.32 mL 1 M LHMDs in THF was added drop wise. After stirred at 0 °C for 30 min. 0.11 mL iodomethane was added drop wise. The resulted mixture was stirred from 0 °C to rt for 2 hour, quenched with 50 mL sat. NH₄Cl, extracted with dichloromethane 3 x 25 mL. The organic phase was dried over anhydrous Na₂SO₄. After purification by flash chromatography, obtained the title compound
25 0.4 g as light solid. MS (ES⁺): 418 (M+H)⁺.

Step C: 1-Cyclohexylmethyl-4-(3,4-dichloro-phenyl)-2-methyl-5-pyridin-4-yl-1,2-dihydro-pyrazol-3-one

The solution of 1-Cyclohexylmethyl-4-(3,4-dichloro-phenyl)-2-methyl-5-pyridin-4-yl-pyrazolidin-3-one 0.16 g in toluene 20 mL and DMF 2 mL was treated with phenyltriethylammonium tribromide 0.22 g at room temperature, The reaction mixture was refluxed for 4 h. The reaction was quenched with sat. Na₂SO₃ 25 mL at 0 °C. The reaction mixture was extracted with dichloromethane, 3 x 25 mL. The combined organic phase was washed with brine, dried over anhydrous Na₂SO₄. After purification by flash chromatography, the title compound was obtained as light yellow solid 80 mg. MS (ES⁺): 416 (M+H)⁺.

Example 20

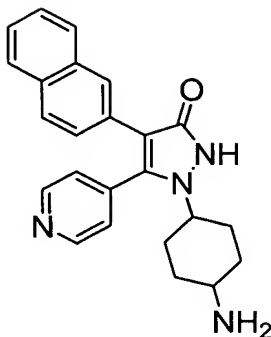
1-(4-Aminocyclohexyl)-4-(4-chlorophenyl)-5-pyridin-4-yl-1,2-dihydropyrazol-3-one

Step A: {4-[4-(4-Chlorophenyl)-3-oxo-5-pyridin-4-yl-2,3-dihydropyrazol-1-yl]cyclohexyl} carbamic acid *tert*-butyl ester

Following the procedure of Step A in Example 5, except substituting 4-oxo-piperidine-1-carboxylic acid *tert*-butyl ester with (4-oxo-cyclohexyl)carbamic acid *tert*-butyl ester, the title compound was prepared as an off-white amorphous solid. MS (ES⁺): 469.2 (M+H)⁺; (ES⁻): 467.3 (M-H)⁻.

Step B: 1-(4-Aminocyclohexyl)-4-(4-chlorophenyl)-5-pyridin-4-yl-1,2-dihydropyrazol-3-one

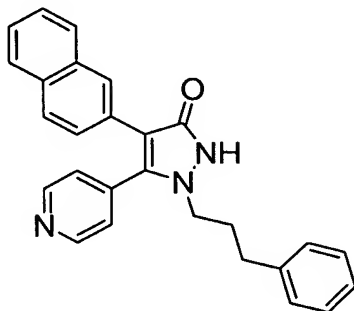
Following the procedure of Step C in Example 5, except substituting 4-[4-(4-chlorophenyl)-3-oxo-5-pyridin-4-yl-2,3-dihydro-pyrazol-1-yl]-piperidine-1-carboxylic acid *tert*-butyl ester with {4-[4-(4-chlorophenyl)-3-oxo-5-pyridin-4-yl-2,3-dihydropyrazol-1-yl]cyclohexyl} carbamic acid *tert*-butyl ester, the title compound was prepared as a yellow amorphous solid. MS (ES⁺): 369.3 (M+H)⁺; (ES⁻): 367.2 (M-H)⁻.

Example 21

1-(4-Aminocyclohexyl)-4-naphthalen-2-yl-5-pyridin-4-yl-1,2-dihydropyrazol-3-one

The title compound was synthesized analogously by the method described in

- 5 Example 20. Naphthalen-2-yl acetic acid, instead of (4-chlorophenyl)acetic acid, was used. MS (ES⁺): 385.2 (M+H)⁺; (ES⁻): 383.3 (M-H)⁻.

Example 22

4-Naphthalen-2-yl-1-(3-phenylpropyl)-5-pyridin-4-yl-1,2-dihydropyrazol-3-one

- 10 The title compound was synthesized analogously by the method described in Step A, Example 20. 3-Phenylpropionaldehyde, instead of 4-oxo-piperidine-1-carboxylic acid *tert*-butyl ester, was used. MS (ES⁺): 390.2 (M+H)⁺; (ES⁻): 388.2 (M-H)⁻.

Biological Assays

- 15 The following assays were used to characterize the ability of compounds of the invention to inhibit the production of TNF- α and IL-1- β . The second assay can be used to measure the inhibition of TNF- α and/or IL-1- β in mice after oral administration of the test compounds. The third assay, a glucagon binding inhibition in vitro assay, can be used to characterize the ability of compounds of the invention to inhibit glucagon binding. The fourth assay, a cyclooxygenase enzyme
- 20 (COX-1 and COX-2) inhibition activity in vitro assay, can be used to characterize the ability of compounds of the invention to inhibit COX-1 and/or COX-2. The fifth

assay, a Raf-kinase inhibition assay, can be used to characterize the compounds of the invention to inhibit phosphorylation of MEK by activated Raf-kinase.

Lipopolysaccharide-activated monocyte TNF production assay

Isolation of monocytes

- 5 Test compounds were evaluated *in vitro* for the ability to inhibit the production of TNF by monocytes activated with bacterial lipopolysaccharide (LPS). Fresh residual source leukocytes (a byproduct of plateletpheresis) were obtained from a local blood bank, and peripheral blood mononuclear cells (PBMCs) were isolated by density gradient centrifugation on Ficol-Paque Plus (Pharmacia).
- 10 PBMCs were suspended at $2 \times 10^6/\text{mL}$ in DMEM supplemented to contain 2% FCS, 10mM, 0.3 mg/mL glutamate, 100 U/mL penicillin G and 100 mg/mL streptomycin sulfate (complete media). Cells were plated into Falcon flat bottom, 96 well culture plates (200 $\mu\text{L}/\text{well}$) and cultured overnight at 37 °C and 6% CO₂. Non-adherent cells were removed by washing with 200 $\mu\text{L}/\text{well}$ of fresh medium. Wells containing
- 15 adherent cells (~70% monocytes) were replenished with 100 μL of fresh medium.

Preparation of test compound stock solutions

- Test compounds were dissolved in DMZ. Compound stock solutions were prepared to an initial concentration of 10 - 50 μM . Stocks were diluted initially to 20 - 200 μM in complete media. Nine two-fold serial dilutions of each compound
- 20 were then prepared in complete medium.

Treatment of cells with test compounds and activation of TNF production with lipopolysaccharide

- One hundred microliters of each test compound dilution were added to microtiter wells containing adherent monocytes and 100 μL complete medium.
- 25 Monocytes were cultured with test compounds for 60 min at which time 25 μL of complete medium containing 30 ng/mL lipopolysaccharide from *E. coli* K532 were added to each well. Cells were cultured an additional 4 hrs. Culture supernatants were then removed and TNF presence in the supernatants was quantified using an ELISA.

30 *TNF ELISA*

 Flat bottom, 96 well Corning High Binding ELISA plates were coated overnight (4 °C) with 150 $\mu\text{L}/\text{well}$ of 3 $\mu\text{g}/\text{mL}$ murine anti-human TNF- α MAbs

(R&D Systems #MAB210). Wells were then blocked for 1 h at room temperature with 200 μ L/well of CaCl_2 -free ELISA buffer supplemented to contain 20 mg/mL BSA (standard ELISA buffer: 20mM, 150mM NaCl, 2mM CaCl_2 , 0.15mM thimerosal, pH 7.4). Plates were washed and replenished with 100 μ L of test
5 supernatants (diluted 1:3) or standards. Standards consisted of eleven 1.5-fold serial dilutions from a stock of 1 ng/mL recombinant human TNF (R&D Systems). Plates were incubated at room temperature for 1 h on orbital shaker (300 rpm), washed and replenished with 100 μ L/well of 0.5 μ g/mL goat anti-human TNF- α (R&D systems #AB-210-NA) biotinylated at a 4:1 ratio. Plates were incubated for 40 min, washed
10 and replenished with 100 μ L/well of alkaline phosphatase-conjugated streptavidin (Jackson ImmunoResearch #016-050-084) at 0.02 μ g/mL. Plates were incubated 30 min, washed and replenished with 200 μ L/well of 1 mg/mL of p-nitrophenyl phosphate. After 30 min, plates were read at 405 nm on a V_{max} plate reader.

Data analysis

15 Standard curve data were fit to a second order polynomial and unknown TNF- α concentrations determined from their OD by solving this equation for concentration. TNF concentrations were then plotted vs. test compound concentration using a second order polynomial. This equation was then used to calculate the concentration of test compounds causing a 50% reduction in TNF
20 production.

Compounds of the invention can also be shown to inhibit LPS-induced release of IL-1 β , IL-6 and/or IL-8 from monocytes by measuring concentrations of IL-1 β , IL-6 and/or IL-8 by methods well known to those skilled in the art. In a similar manner to the above described assay involving the LPS induced release of
25 TNF- α from monocytes, compounds of this invention can also be shown to inhibit LPS induced release of IL-1 β , IL-6 and/or IL-8 from monocytes by measuring concentrations of IL-1 β , IL-6 and/or IL-8 by methods well known to those skilled in the art. Thus, the compounds of the invention may lower elevated levels of TNF- α , IL-1, IL-6, and IL-8 levels. Reducing elevated levels of these inflammatory
30 cytokines to basal levels or below is favorable in controlling, slowing progression, and alleviating many disease states. All of the compounds are useful in the methods

of treating disease states in which TNF- α , IL-1 β , IL-6, and IL-8 play a role to the full extent of the definition of TNF- α -mediated diseases described herein.

Lipopolysaccharide-activated THP1 Cell TNF production assay

THP1 cells are resuspended in fresh THP1 media (RPMI 1640, 10% heat-inactivated FBS, 1XPGS, 1XNEAA, plus 30 μ M β ME) at a concentration of 1E6/mL. One hundred microliters of cells per well are plated in a polystyrene 96-well tissue culture. One microgram per mL of bacterial LPS is prepared in THP1 media and is transferred to the wells. Test compounds are dissolved in 100% DMSO and are serially diluted 3 fold in a polypropylene 96-well microtiter plate (drug plate). HI control and LO control wells contain only DMSO. One microliter of test compound from the drug plate followed by 10 μ L of LPS are transferred to the cell plate. The treated cells are induced to synthesize and secrete TNF- α at 37 $^{\circ}$ C for 3 h. Forty microliters of conditioned media are transferred to a 96-well polypropylene plate containing 110 μ L of ECL buffer (50mM Tris-HCl pH 8.0, 100mM NaCl, 0.05% Tween 20, 0.05% NaN₃ and 1%FBS) supplemented with 0.44nM MAB610 monoclonal Ab (R&D Systems), 0.34nM ruthenylated AF210NA polyclonal Ab (R&D Systems) and 44 μ g/mL sheep anti-mouse M280 Dynabeads (Dyna). After a 2 h incubation at room temperature with shaking, the reaction is read on the ECL M8 Instrument (IGEN Inc.). A low voltage is applied to the ruthenylated TNF- α immune complexes, which in the presence of TPA (the active component in Origlo), results in a cyclical redox reaction generating light at 620nm. The amount of secreted TNF- α in the presence of compound compared with that in the presence of DMSO vehicle alone (HI control) is calculated using the formula: % control (POC) = (cpd – average LO)/(average HI - average LO)*100. Data (consisting of POC and inhibitor concentration in μ M) is fitted to a 4-parameter equation ($y = A + ((B-A)/(1 + ((x/C)^D)))$), where A is the minimum y (POC) value, B is the maximum y (POC), C is the x (cpd concentration) at the point of inflection and D is the slope factor) using a Levenburg-Marquardt non-linear regression algorithm.

The following compounds exhibit activities in the THP1 cell assay (LPS induced TNF release) with IC₅₀ values of 20 μ M or less:

- 4-(4-chloro-phenyl)-1-piperidin-4-yl-5-pyridin-4-yl-1,2-dihydro-pyrazol-3-one;
4-(4-chloro-phenyl)-1-piperidin-3-yl-5-pyridin-4-yl-1,2-dihydro-pyrazol-3-one;
4-(4-chlorophenyl)-1-(1-methyl-piperidin-4-yl)-5-pyridin-4-yl-1,2-dihydro-pyrazol-3-one;
5 4-(4-chlorophenyl)-1-(1-methyl-piperidin-3-yl)-5-pyridin-4-yl-1,2-dihydro-pyrazol-3-one;
4-(4-chlorophenyl)-1-(1-isopropyl-piperidin-4-yl)-5-pyridin-4-yl-1,2-dihydro-pyrazol-3-one;
4-(3,4-dichloro-phenyl)-5-pyridin-4-yl-1,2-dihydro-pyrazol-3-one;
10 4-(3,4-dichloro-phenyl)-1-isopropyl-5-pyridin-4-yl-1,2-dihydro-pyrazol-3-one;
4-(3,4-dichloro-phenyl)-1-isopropyl-2-methyl-5-pyridin-4-yl-1,2-dihydro-pyrazol-3-one;
1-cyclohexylmethyl-4-(3,4-dichloro-phenyl)-2-methyl-5-pyridin-4-yl-1,2-dihydro-pyrazol-3-one; and
15 1-(4-aminocyclohexyl)-4-(4-chlorophenyl)-5-pyridin-4-yl-1,2-dihydro-pyrazol-3-one.

Inhibition of LPS-Induced TNF- α production in mice

- Male DBA/1LACJ mice are dosed with vehicle or test compounds in a vehicle (the vehicle consisting of 0.5% tragacanth in 0.03 N HCl) 30 minutes prior to lipopolysaccharide (2 mg/Kg, I.V.) injection. Ninety minutes after LPS injection,
20 blood is collected and the serum is analyzed by ELISA for TNF- α levels.

- Compounds of the invention may be shown to have anti-inflammatory properties in animal models of inflammation, including carrageenan paw edema, collagen induced arthritis and adjuvant arthritis, such as the carrageenan paw edema model (C. A. Winter et al Proc. Soc. Exp. Biol. Med. (1962) vol 111, p 544; K. F. Swingle, in R. A. Scherrer and M. W. Whitehouse, Eds., Anti-inflammatory Agents, Chemistry and Pharmacology, Vol. 13-II, Academic, New York, 1974, p. 33) and collagen induced arthritis (D. E. Trentham et al J. Exp. Med. (1977) vol. 146, p 857; J. S. Courtenay, Nature (New Biol.) (1980), Vol 283, p 666).

¹²⁵I-Glucagon Binding Screen with CHO/hGLUR Cells

- 30 The assay is described in WO 97/16442, which is incorporated herein by reference in its entirety.

Reagents

The reagents can be prepared as follows: (a) prepare fresh 1M o-Phenanthroline (Aldrich) (198.2 mg/mL ethanol); (b) prepare fresh 0.5M DTT (Sigma); (c) Protease Inhibitor Mix (1000X): 5 mg leupeptin, 10 mg benzamidine, 40 mg bacitracin and 5 mg soybean trypsin inhibitor per mL DMSO and store aliquots at -20 °C; (d) 250 µM human glucagon (Peninsula): solubilize 0.5 mg vial in 575 µl 0.1N acetic acid (1 µL yields 1 µM final concentration in assay for non-specific binding) and store in aliquots at -20 °C; (e) Assay Buffer: 20mM Tris (pH 7.8), 1mM DTT and 3mM o-phenanthroline; (f) Assay Buffer with 0.1% BSA (for dilution of label only; 0.01% final in assay): 10 µL 10% BSA (heat-inactivated) and 990 µL Assay Buffer; (g) ¹²⁵I-Glucagon (NEN, receptor-grade, 2200 Ci/mmol): dilute to 50,000 cpm/25 µL in assay buffer with BSA (about 50pM final concentration in assay).

Harvesting of CHO/hGLUR Cells for Assay

1. Remove media from confluent flask then rinse once each with PBS (Ca, Mg-free) and Enzyme-free Dissociation Fluid (Specialty Media, Inc.).
2. Add 10 mL Enzyme-free Dissoc. Fluid and hold for about 4 min at 37 °C.
3. Gently tap cells free, triturate, take aliquot for counting and centrifuge remainder for 5 min at 1000 rpm.
4. Resuspend pellet in Assay Buffer at 75000 cells per 100 µL.

Membrane preparations of CHO/hGLUR cells can be used in place of whole cells at the same assay volume. Final protein concentration of a membrane preparation is determined on a per batch basis.

Assay

- The determination of inhibition of glucagon binding can be carried out by measuring the reduction of ¹²⁵I-glucagon binding in the presence of compounds of Formula I. The reagents are combined as follows:

	Compound/ Vehicle	250µM Glucagon	¹²⁵ I-Glucagon	CHO/hGLUR Cells
Total Binding	--/5 µL	--	25 µL	100 µL
+ Compound	5 µL/--	--	25 µL	100 µL

Nonspecific		--/5 μ L	1 μ L	25 μ L	100 μ L
Binding					

The mixture is incubated for 60 min at 22 °C on a shaker at 275 rpm. The mixture is filtered over pre-soaked (0.5% polyethylimine (PEI)) GF/C filtermat using an Innotech Harvester or Tomtec Harvester with four washes of ice-cold 20mM Tris buffer (pH 7.8). The radioactivity in the filters is determined by a gamma-scintillation counter.

Thus, compounds of the invention may also be shown to inhibit the binding of glucagon to glucagon receptors.

Cyclooxygenase Enzyme Activity Assay

The human monocytic leukemia cell line, THP-1, differentiated by exposure to phorbol esters expresses only COX-1; the human osteosarcoma cell line 143B expresses predominantly COX-2. THP-1 cells are routinely cultured in RPMI complete media supplemented with 10% FBS and human osteosarcoma cells (HOSC) are cultured in minimal essential media supplemented with 10% fetal bovine serum (MEM-10%FBS); all cell incubations are at 37 °C in a humidified environment containing 5% CO₂.

COX-1 Assay

In preparation for the COX-1 assay, THP-1 cells are grown to confluency, split 1:3 into RPMI containing 2% FBS and 10mM phorbol 12-myristate 13-acetate (TPA), and incubated for 48 h on a shaker to prevent attachment. Cells are pelleted and resuspended in Hank's Buffered Saline (HBS) at a concentration of 2.5×10^6 cells/mL and plated in 96-well culture plates at a density of 5×10^5 cells/mL. Test compounds are diluted in HBS and added to the desired final concentration and the cells are incubated for an additional 4 hours. Arachidonic acid is added to a final concentration of 30mM, the cells incubated for 20 minutes at 37 °C, and enzyme activity determined as described below.

COX-2 Assay

For the COX-2 assay, subconfluent HOSC are trypsinized and resuspended at 3×10^6 cells/mL in MEM-FBS containing 1 ng human IL-1b/mL, plated in 96-

well tissue culture plates at a density of 3×10^4 cells per well, incubated on a shaker for 1 hour to evenly distribute cells, followed by an additional 2 hour static incubation to allow attachment. The media is then replaced with MEM containing 2% FBS (MEM-2%FBS) and 1 ng human IL-1b/mL, and the cells incubated for 18-22 hours. Following replacement of media with 190 mL MEM, 10 mL of test compound diluted in HBS is added to achieve the desired concentration and the cells incubated for 4 hours. The supernatants are removed and replaced with MEM containing 30mM arachidonic acid, the cells incubated for 20 minutes at 37 °C, and enzyme activity determined as described below.

10 COX Activity Determined

After incubation with arachidonic acid, the reactions are stopped by the addition of 1N HCl, followed by neutralization with 1N NaOH and centrifugation to pellet cell debris. Cyclooxygenase enzyme activity in both HOSC and THP-1 cell supernatants is determined by measuring the concentration of PGE₂ using a commercially available ELISA (Neogen #404110). A standard curve of PGE₂ is used for calibration, and commercially available COX-1 and COX-2 inhibitors are included as standard controls.

Raf Kinase assay

In vitro Raf kinase activity is measured by the extent of phosphorylation of the substrate MEK (Map kinase/ERK kinase) by activated Raf kinase, as described in GB 1,238,959 (incorporated herein by reference in its entirety). Phosphorylated MEK is trapped on a filter and incorporation of radiolabeled phosphate is quantified by scintillation counting.

MATERIALS:

25 Activated Raf is produced by triple transfection of Sf9 cells with baculoviruses expressing “Glu-Glu”-epitope tagged Raf, val¹²-H-Ras, and Lck. The “Glu-Glu”-epitope, Glu-Try-Met-Pro-Met-Glu, was fused to the carboxy-terminus of full length c-Raf.

Catalytically inactive MEK (K97A mutation) is produced in Sf9 cells transfected with a baculovirus expressing c-terminus “Glu-Glu” epitope-tagged K97A MEK1.

Anti “Glu-Glu” antibody was purified from cells grown as described in:

Grussenmeyer, et al., Proceedings of the National Academy of Science, U.S.A. pp 7952-7954, 1985.

Column buffer: 20mM Tris pH 8, 100mM NaCl, 1mM EDTA, 2.5mM EGTA, 10mM
5 MgCl₂, 2mM DTT, 0.4mM AEBSF, 0.1% n-octylglucopyranoside, 1nM okadaic acid,
and 10 µg/mL each of benzamidine, leupeptin, pepstatin, and aprotinin.

5x Reaction buffer: 125mM HEPES pH=8, 25mM MgCl₂, 5mM EDTA, 5mM
Na₃VO₄, 100 µg/mL BSA.

Enzyme dilution buffer: 25mM HEPES pH 8, 1mM EDTA, 1mM Na₃VO₄,
10 400 µg/mL BSA.

Stop solution: 100mM EDTA, 80mM sodium pyrophosphate.

Filter plates: Milipore multiscreen # SE3MO78E3, Immobilon-P (PVDF).

METHODS:

Protein purification: Sf9 cells were infected with baculovirus and grown as
15 described in Williams, et al., Proceedings of the National Academy of Science,
U.S.A. pp 2922-2926, 1992. All subsequent steps were performed on ice or at
4 °C. Cells were pelleted and lysed by sonication in column buffer. Lysates were
spun at 17,000xg for 20 min, followed by 0.22 µm filtration. Epitope tagged
proteins were purified by chromatography over GammaBind Plus affinity column to
20 which the “Glu-Glu” antibody was coupled. Proteins were loaded on the column
followed by sequential washes with two column volumes of column buffer, and
eluted with 50 µg/mL Glu-Tyr-Met-Pro-Met-Glu in column buffer.

Raf kinase assay: Test compounds were evaluated using ten 3-fold serial dilutions
starting at 10 - 100µM. 10 µL of the test inhibitor or control, dissolved in 10%
25 DMSO, was added to the assay plate followed by the addition of 30 µL of the a
mixture containing 10 µL 5x reaction buffer, 1mM ³³P-γ-ATP (20 µCi/mL), 0.5 µL
MEK (2.5 mg/mL), 1 µL 50mM β-mercaptoethanol. The reaction was started by the
addition of 10 µL of enzyme dilution buffer containing 1mM DTT and an amount of
activated Raf that produces linear kinetics over the reaction time course. The
30 reaction was mixed and incubated at room temperature for 90 min and stopped by
the addition of 50 µL stop solution. 90 µL aliquots of this stopped solution were

transferred onto GFP-30 cellulose microtiter filter plates (Polyfiltronics), the filter plates washed in four well volumes of 5% phosphoric acid, allowed to dry, and then replenished with 25 μ L scintillation cocktail. The plates were counted for ^{33}P gamma emission using a TopCount Scintillation Reader.

5 While the compounds of the invention can be administered as the sole active pharmaceutical agent, they can also be used in combination with one or more compounds of the invention or other agents. When administered as a combination, the therapeutic agents can be formulated as separate compositions that are given at the same time or different times, or the therapeutic agents can be given as a single
10 composition.

The foregoing is merely illustrative of the invention and is not intended to limit the invention to the disclosed compounds. Variations and changes which are obvious to one skilled in the art are intended to be within the scope and nature of the invention which are defined in the appended claims.

15 From the foregoing description, one skilled in the art can easily ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions.

20 For the treatment of TNF- α , IL-1 β , IL-6, and IL-8 mediated diseases, cancer, and/or hyperglycemia, the compounds of the present invention may be administered orally, parentally, by inhalation spray, rectally, or topically in dosage unit formulations containing conventional pharmaceutically acceptable carriers, adjuvants, and vehicles. The term parenteral as used herein includes, subcutaneous, intravenous, intramuscular, intrasternal, infusion techniques or intraperitoneally.

25 Treatment of diseases and disorders herein is intended to also include the prophylactic administration of a compound of the invention, a pharmaceutical salt thereof, or a pharmaceutical composition of either to a subject (*i.e.*, an animal, preferably a mammal, most preferably a human) believed to be in need of preventative treatment, such as, for example, pain, inflammation and the like.

30 The dosage regimen for treating a TNF- α , IL-1, IL-6, and IL-8 mediated diseases, cancer, and/or hyperglycemia with the compounds of this invention and/or

compositions of this invention is based on a variety of factors, including the type of disease, the age, weight, sex, medical condition of the patient, the severity of the condition, the route of administration, and the particular compound employed.

Thus, the dosage regimen may vary widely, but can be determined routinely using standard methods. Dosage levels of the order from about 0.01 mg to 30 mg per kilogram of body weight per day, preferably from about 0.1 mg to 10 mg/kg, more preferably from about 0.25 mg to 1 mg/kg are useful for all methods of use disclosed herein.

The pharmaceutically active compounds of this invention can be processed in accordance with conventional methods of pharmacy to produce medicinal agents for administration to patients, including humans and other mammals.

For oral administration, the pharmaceutical composition may be in the form of, for example, a capsule, a tablet, a suspension, or liquid. The pharmaceutical composition is preferably made in the form of a dosage unit containing a given amount of the active ingredient. For example, these may contain an amount of active ingredient from about 1 to 2000 mg, preferably from about 1 to 500 mg, more preferably from about 5 to 150 mg. A suitable daily dose for a human or other mammal may vary widely depending on the condition of the patient and other factors, but, once again, can be determined using routine methods.

The active ingredient may also be administered by injection as a composition with suitable carriers including saline, dextrose, or water. The daily parenteral dosage regimen will be from about 0.1 to about 30 mg/kg of total body weight, preferably from about 0.1 to about 10 mg/kg, and more preferably from about 0.25 mg to 1 mg/kg.

Injectable preparations, such as sterile injectable aqueous or oleaginous suspensions, may be formulated according to the known art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally acceptable diluent or solvent, for example as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this

purpose any bland fixed oil may be employed, including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables.

Suppositories for rectal administration of the drug can be prepared by
5 mixing the drug with a suitable non-irritating excipient such as cocoa butter and polyethylene glycols that are solid at ordinary temperatures but liquid at the rectal temperature and will therefore melt in the rectum and release the drug.

A suitable topical dose of active ingredient of a compound of the invention is 0.1 mg to 150 mg administered one to four, preferably one or two times daily.

10 For topical administration, the active ingredient may comprise from 0.001% to 10% w/w, *e.g.*, from 1% to 2% by weight of the formulation, although it may comprise as much as 10% w/w, but preferably not more than 5% w/w, and more preferably from 0.1% to 1% of the formulation.

Formulations suitable for topical administration include liquid or semi-liquid
15 preparations suitable for penetration through the skin (*e.g.*, liniments, lotions, ointments, creams, or pastes) and drops suitable for administration to the eye, ear, or nose.

For administration, the compounds of this invention are ordinarily combined with one or more adjuvants appropriate for the indicated route of administration.

20 The compounds may be admixed with lactose, sucrose, starch powder, cellulose esters of alkanolic acids, stearic acid, talc, magnesium stearate, magnesium oxide, sodium and calcium salts of phosphoric and sulphuric acids, acacia, gelatin, sodium alginate, polyvinyl-pyrrolidone, and/or polyvinyl alcohol, and tableted or encapsulated for conventional administration. Alternatively, the compounds of this
25 invention may be dissolved in saline, water, polyethylene glycol, propylene glycol, ethanol, corn oil, peanut oil, cottonseed oil, sesame oil, tragacanth gum, and/or various buffers. Other adjuvants and modes of administration are well known in the pharmaceutical art. The carrier or diluent may include time delay material, such as glyceryl monostearate or glyceryl distearate alone or with a wax, or other materials
30 well known in the art.

The pharmaceutical compositions may be made up in a solid form (including granules, powders or suppositories) or in a liquid form (*e.g.*, solutions, suspensions,

or emulsions). The pharmaceutical compositions may be subjected to conventional pharmaceutical operations such as sterilization and/or may contain conventional adjuvants, such as preservatives, stabilizers, wetting agents, emulsifiers, buffers etc.

- Solid dosage forms for oral administration may include capsules, tablets, pills, powders, and granules. In such solid dosage forms, the active compound may be admixed with at least one inert diluent such as sucrose, lactose, or starch. Such dosage forms may also comprise, as in normal practice, additional substances other than inert diluents, *e.g.*, lubricating agents such as magnesium stearate. In the case of capsules, tablets, and pills, the dosage forms may also comprise buffering agents.
- 10 Tablets and pills can additionally be prepared with enteric coatings.

Liquid dosage forms for oral administration may include pharmaceutically acceptable emulsions, solutions, suspensions, syrups, and elixirs containing inert diluents commonly used in the art, such as water. Such compositions may also comprise adjuvants, such as wetting, sweetening, flavoring, and perfuming agents.